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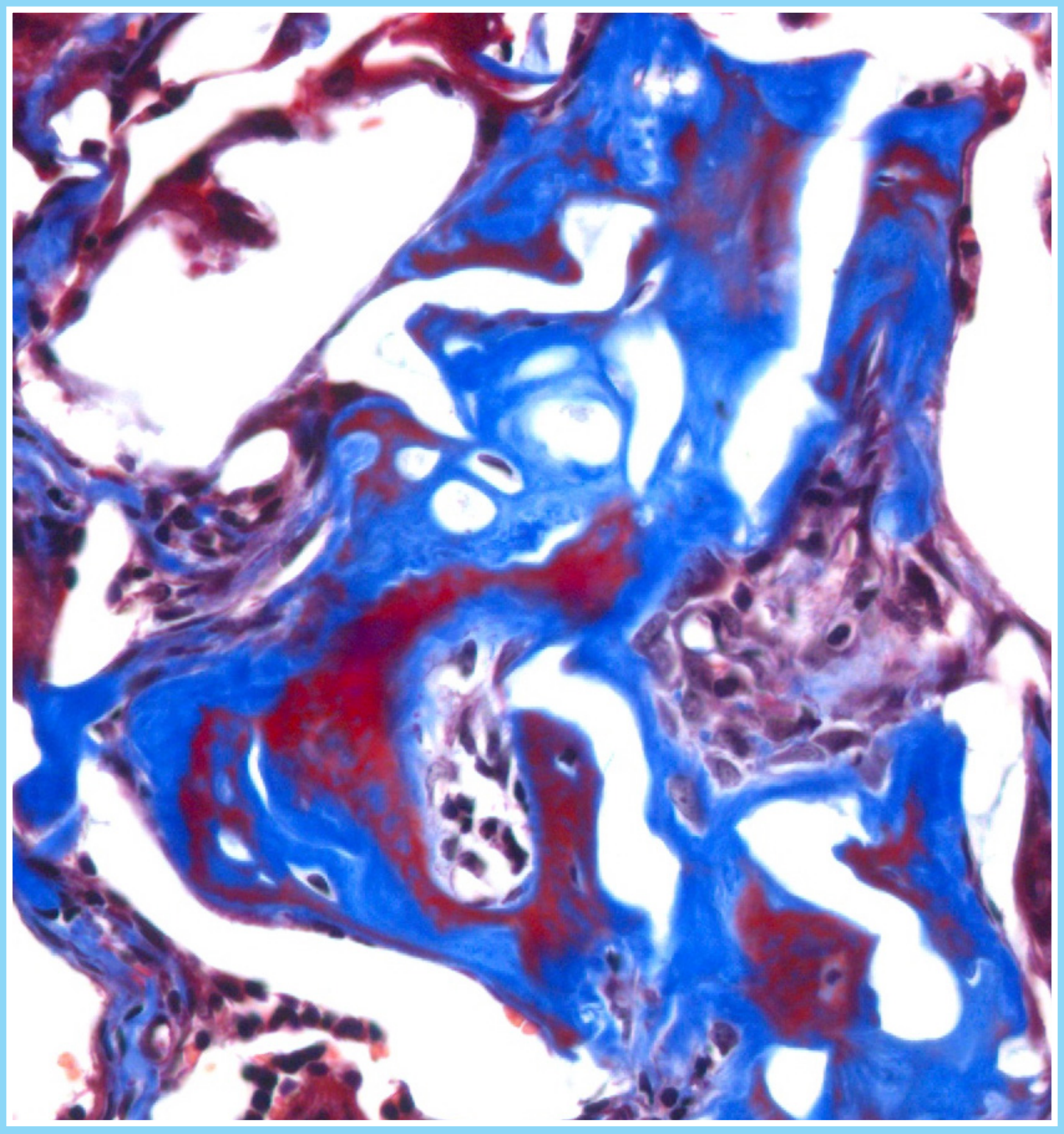

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Authors: Alberto González-González[1], Ricardo Reyes[2], Flor M. Pérez Campo[1]
[1]Department of Molecular Biology. Faculty of Medicine. Universidad de Cantabria-IDIVAL, Santander. [2]Department of Biochemistry, Microbiology, Cellular Biology, and Genetics. Institute of Biomedical Technologies. Universidad de La Laguna. La Laguna, Tenerife

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Original

Association of gamma glutamyl transferase in the presence and progression of abdominal aortic calcifications and changes to bone mineral density

Beatriz Martín Carro[1,3], Carlos Gómez Alonso[1], Minerva Rodríguez García[2,3], Noelia Avello Llano[4], Carmen García Gil-Albert[4], Lucía Sobrino Díaz[2], Francisco Baena Huerta[1], Carmen Palomo Antequera[5], Laura Naves Mendívil[1], Javier Rodríguez Carrio[6], José Luis Fernández Martín[1,3], Manuel Naves Díaz[1,3]

[1]Clinical Management Unit of Bone Metabolism. Hospital Universitario Central de Asturias. Universidad de Oviedo. Instituto de Investigación Sanitaria del Principado de Asturias (ISPA). Oviedo, Spain. [2]Clinical Management Area of Nephrology. Hospital Universitario Central de Asturias. Universidad de Oviedo. Instituto de Investigación Sanitaria del Principado de Asturias (ISPA). Oviedo, Spain. [3]REDinREN del ISCIII. [4]Laboratory of Medicine. Hospital Universitario Central de Asturias. Oviedo, Spain. [5]Clinical Management Unit of Internal Medicine. Hospital Universitario Central de Asturias. Universidad de Oviedo. ISPA. Oviedo, Spain. [6]Department of Functional Biology. Investigación Básica y Traslacional en Enfermedades inflamatorias Crónicas. Universidad de Oviedo. ISPA. Oviedo, Spain

Abstract

Introduction and objective: abdominal aortic calcification (AAC) is a predictor of cardiovascular events. This study aimed to assess the association of gamma glutamyl transferase (GGT) in the presence and progression of AAC, as well as changes to bone mineral density (BMD) in the lumbar spine and femoral neck.

Materials and methods: a total of 326 men and women over 50 years of age were selected for this study. They completed a questionnaire, underwent two lateral dorso-lumbar spine X-rays, and BMD measurements. The same tests and 1 analytical assessment were repeated after 4 years.

Results: the presence and progression of AAC (new occurrences or increased severity) were lower in GGT quartile 1 (Q1) compared with the other quartiles (40 % vs 58 %; p = 0.021; 24 % vs 44 %; p = 0.022). Compared with Q1, the confounders-adjusted logistic regression analysis showed that Q2 and Q4 were associated with more presence of AAC [odds ratio (OR), 2.53; 95 % confidence interval (95 % CI), 1.22-5.25 and OR, 3.04; 95 % CI, 1.36-6.77]. Additionally, Q2, Q3, and Q4 were associated with more AAC progression [OR, 2.24; 95 % CI, 1.07-4.67; OR, 2.35; 95 % CI, 1.09-5.07; and OR, 3.47; 95 % CI, 1.56-7.70]. The gender-stratified multivariate analysis revealed that in both men and women, the Q4 of GGT was associated with AAC progression [OR, 3.27; 95 % CI, 1.14-9.36, and OR, 3.26; 95 % CI, 1.03-10.29, respectively], and in women alone, with greater lumbar BMD losses. There were no effects regarding the prevalence of AAC.

Conclusions: elevated GGT levels could serve as an indicator of the presence and progression of AAC in individuals older than 50 years. When analyzed separately by gender, higher GGT levels were associated with AAC progression, which acted as a prognostic marker for cardiovascular disease.

Keywords: Gamma glutamyl transferase. Abdominal aortic calcification. Overall population. Bone mineral density.

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Correspondence:
Manuel Naves Díaz. Unidad de Gestión Clínica de Metabolismo Óseo. Hospital Universitario Central de Asturias. Avenida de Roma, s/n. 33011 Oviedo, Spain
e-mail: mnaves.huca@gmail.com

INTRODUCTION

Vascular calcification is a process wherein vascular smooth muscle cells dedifferentiate into osteoblasts, resulting in the formation of bone in inappropriate locations. Vascular calcification is a significant issue of public health that is projected to escalate due to the aging population. Data from the European study on vertebral osteoporosis estimated a prevalence of abdominal aortic calcification in the overall population over 50 years old of 38 %, being more common in men (46 %) than women (30 %) (1). The abdominal aorta is one of the first vascular beds where atherosclerotic calcification can be seen, often preceding the development of coronary artery calcification (2,3). Abdominal aortic calcification contributes to arterial stiffness and strongly predicts cardiovascular events and mortality (4).

Numerous classical factors have been associated with abdominal aortic calcification, including age, hypertension, smoking, hyperlipidemia, diabetes *mellitus,* and overweight, among others. However, using one single biomarker to assess the presence of abdominal aortic calcification before its consequences start to show is still challenging. Gamma glutamyl transferase (GGT), an enzyme used as a marker of hepatobiliary diseases and alcohol consumption, is also recognized as a true marker of atherosclerotic disease. Former studies have demonstrated that serum GGT levels, even within normal ranges, are associated with atherosclerotic risk factors and predict the future occurrence of cardiovascular diseases, hypertension, stroke, metabolic syndrome, and type 2 diabetes (5-8).

A meta-analysis proved the existence of a relationship between GGT and the incidence of cardiovascular events regardless of alcohol consumption (9). While various studies have explored coronary aortic calcification and serum GGT levels in cross-sectional cohorts (10-13), we still need studies that other from confirming these findings in the abdominal aorta and within our geographic context, can prove that there is a potentially stronger association than the one found in the previously mentioned cross-sectional studies. Therefore, the objective of this study was to assess the predictive potential of GGT in the prevalence and progression of abdominal aortic calcification, and in changes to the lumbar spine and femoral neck bone mineral density (BMD) in an unselected overall population of men and women older than 50 years.

MATERIAL AND METHODS

This study was conducted using data from a European project aimed at determining the prevalence of vertebral fractures (European vertebral osteoporosis study - EVOS) (14) with participation from the Bone Metabolism Clinical Unit of Hospital Universitario Central de Asturias in Oviedo, Spain.

A random selection was made from the Oviedo municipal registry including 308 men and 316 women older than 50 years. The study protocol involved the completion of a questionnaire on osteoporosis-related risk factors, 2 lateral dorso-lumbar spine X-rays (the radiographic study was only incomplete in 2 cases), and the collection of anthropometric measurements such as height and weight to determine the body mass index (BMI). All participants had enough walking capabilities to climb 2 flights of stairs without an elevator, and 99 % of them lived in their own homes.

After 4 years, participants were invited to undergo the same radiographic study, anthropometric measurements, a questionnaire on osteoporosis risk factors, and a biochemical analysis. In the second follow-up, a total of 402 participants (213 women and 189 men) were included. The data of 326 subjects were available at the beginning of the study and 4 years later.

ASSESSMENT OF PROGRESSION OF VASCULAR CALCIFICATION

Abdominal aortic calcification was assessed by two independent investigators, defined, and categorized into 3 grades: grade 0 (absent), grade 1 (mild-to-moderate), and grade 2 (severe). Isolated punctate calcifications, a visible linear calcification spanning fewer than 2 vertebral bodies, or a dense calcified plaque were defined as mild-to-moderate calcification (1). The presence of a visible linear calcification extending across, at least, 2 vertebral bodies and/or the presence of 2 or more dense calcified plaques were categorized as severe calcification. The degree of intra- and inter-observer agreement for the radiographic analysis was 92 % and 90 %, respectively, with a Kappa coefficient of 0.78 and 0.73, indicative of good reproducibility (1).

The progression of aortic calcification was determined by comparing the X-rays taken at the beginning of the study with those taken 4 years later. Aortic calcification "progression" was defined as an increase in the extent of baseline aortic calcification along with the appearance of new calcifications, as seen in the comparison between the early x-rays and those taken 4 years later.

DENSITOMETRIC EVALUATION

BMD was measured using a Hologic® QDR-1000 DXA densitometer (Hologic Inc., Waltham, MA, United States). In all cases, measurements were taken of the antero-posterior lumbar spine (L2-L4) and right femoral neck. Regarding lumbar BMD assessment, a total of

4 participants with significant degenerative arthritis were excluded. The coefficients of variation (CV) were 1.2 % and 1.9 % respectively (1). Precision and quality control were kept daily using a lumbar spine phantom that yielded a CV of 0.0 ± 0.1 %. In the fourth year, BMD of the same areas was determined as in the early study, and the percentage change between the 2 measurements was used to assess changes to BMD.

BIOCHEMICAL ANALYSIS

During the baseline study, no biochemical analysis was ever conducted. After 4 years, fasting blood and urine samples were collected from each study participant. Once serum and urine samples were separated, they were frozen at -80 °C until quantification. Calcium, creatinine, phosphorus, total alkaline phosphatase, gamma glutamyl transferase (GGT), and serum tartrate-resistant acid phosphatase levels were measured using an autoanalyzer (Hitachi Mod. 717, Ratigen, Germany). The serum levels of calcidiol (25OHD) were determined through prior extraction with acetonitrile (IDS, Ltd., Bolton, United Kingdom), with intra- and inter-assay coefficients of variation (CV) of 5.2 % and 8.2 % respectively.

The levels of 1,25-dihydroxyvitamin D were measured using a radioimmunoassay (IDS, Ltd.), with intra- and inter-assay CVs of 6.5 % and 9 % respectively. Intact PTH and osteocalcin levels were measured using a radioimmunoassay (Nichols Institute, San Juan Capistrano, CA, United States). The intra- and inter-assay CVs were 2.6 % and 5.8 % for PTH, and 4.5 % and 5.1 % for osteocalcin, respectively.

All the studies conducted observed the principles outlined in the Declaration of Helsinki and were formally approved by the Clinical Trials Committee of the Principado de Asturias, Spain.

STATISTICAL ANALYSIS

Data analysis was conducted using version 25.0 of SPSS for Windows. Quantitative variables were analyzed using the Student t test for normally distributed variables and the Mann-Whitney U test for those following abnormal distributions. Qualitative variables were analyzed using the chi-square test.

Multivariate analysis of GGT quartiles was performed on the presence of abdominal aortic calcification, and with progression and/or appearance of new abdominal aortic calcification using logistic regression adjusted for age, sex, BMI, smoking habit, and alcohol intake.

Similarly, a multivariate analysis was performed for the highest GGT levels (quartile 4, while the remaining 3 quartiles were grouped in 1) associated with changes to BMD using linear logistic regression adjusted for age, sex, BMI, smoking habit, and alcohol intake.

RESULTS

Table I shows the clinical characteristics, anthropometric data, and biochemical values differentiated by gender. The mean age was similar (68 years) with higher BMI reported in women. In men, BMD at the lumbar spine and femoral neck, the smoking habit, and weekly alcohol intake > 7 units of alcohol was significantly higher compared with women, as well as the prevalence and progression of abdominal aortic calcification. Among the biochemical parameters, men exhibited more elevated levels of serum creatinine, calcidiol, and GGT. Conversely, women had higher serum levels of phosphorus, PTH, and osteocalcin.

To categorize any potential effect of GGT on abdominal aortic calcification, serum GGT levels were categorized into quartiles. Overall, the presence and progression of abdominal aortic calcification (new AAC or increased severity compared to baseline) were significantly lower in the lowest GGT quartile compared with the remaining quartiles (40 % vs 58 %; $p = 0.021$; 24 % vs 44 %, $p = 0.022$) (Table II). Compared with the lowest GGT quartile (reference), the logistic regression analysis adjusted for age, BMI, sex, smoking habit, and alcohol intake showed that Q2 and Q4 were associated with the presence of more abdominal aortic calcification [OR, 2.53; 95 % confidence interval (95 % CI), 1.22-5.25], and OR, 3.04; 95 % CI, 1.36-6.77, and that Q2, Q3, and Q4 were associated with more abdominal aortic calcification progression [OR, 2.24; 95 % CI, 1.07-4.67; OR, 2.35; 95 % CI, 1.09-5.07; and OR, 3.47; 95 % CI, 1.56-7.70].

Considering the potential effect of alcohol consumption on the GGT levels, associations were analyzed separately based on gender. In the univariate analysis, there was no clear trend in the prevalence and progression of abdominal aortic calcification based on GGT quartiles (Table III). However, the logistic regression analysis conducted separately by gender and adjusted for age, BMI, smoking habit, and alcohol intake, revealed that in both men and women, higher GGT values (Q4) were associated with the progression of abdominal aortic calcification [OR, 3.27; 95 % CI, 1.14-9.36] and OR, 3.26; 95 % CI, 1.03-10.29, respectively. There was no effect reported on the prevalence of abdominal aortic calcification.

Since the levels of GGT were independently associated with the progression of abdominal aortic calcification in both men and women and considering the association between the process of calcification and bone demineralization, changes to BMD between the 2 visits were studied. It was seen that, at lumbar level,

and only in women, higher serum GGT levels (Q4) were associated with greater BMD losses compared to the remaining 3 GGT quartiles grouped together (Table IV). The linear regression analysis adjusted for age, BMI, smoking habit, and alcohol intake showed that the quartile with the highest GGT serum levels was significantly associated with changes to lumbar BMD (standardized beta coefficient of 0.245; p = 0.004). There were no significant differences found in women's femoral neck, although the trend was similar (p = 0.090). In men, no effects were seen in the 2 bone segments analyzed (Table IV). With the remaining bone segments available (trochanter, total hip, or Ward's triangle), no associations with serum GGT levels were found in men or women (data not available).

Table I. Clinical, anthropometric, and biochemical markers of bone and mineral metabolism between men and women

Variables	Men (n = 160)	Women (n = 166)	p-value
Age (years)	68 ± 9	68 ± 8	0.526
BMI (kg/cm^2)	27.7 ± 3.5	28.6 ± 4.5	*0.048*
Lumbar BMD (g/cm^2)	1.020 ± 0.159	0.860 ± 0.155	*< 0.001*
Hip BMD (g/cm^2)	0.804 ± 0.122	0.694 ± 0.116	*< 0.001*
Alcohol > 7 units/week (N/%)	92 (57.5 %)	15 (9.4 %)	*< 0.001*
Current Smokers (N/%)	51 (32.0 %)	7 (4.4 %)	*< 0.001*
Prevalence of AAC (N/%)	96 (60.0 %)	66 (39.8 %)	*< 0.001*
Progression of AAC (N/%)	68 (42.5 %)	52 (31.3 %)	*0.022*
Calcium (mg/dL)	9.41 ± 0.34	9.40 ± 0.36	*0.687*
Phosphorus (mg/dL)	3.31 ± 0.46	3.61 ± 0.42	*< 0.001*
Creatinine (mg/dL)	1.10 (1.01-1.21)	0.90 (0.684-1.00)	*< 0.001*
PTH (pg/mL)	44 (36-60)	52 (38-66)	*0.014*
Calcidiol (ng/mL)	17 (11-23)	13 (9-20)	*< 0.001*
Osteocalcin (ng/mL)	5.1 (4.0-6.1)	6.3(4.9-7.7)	*0.007*
GGT (IU/L)	23.5 (16.2-34.0)	15 (11.7-22.2)	*< 0.001*

Table II. Prevalence and progression of abdominal aortic calcification based on the levels of GGT in quartiles in the overall population

Serum levels of gamma glutamyl transferase (GGT) in IU/L					
	Q1 (< 13)	Q2 (13-18)	Q3 (18-29.25)	Q4 (> 29.25)	p-value
Prevalence of AAC (N/%)	36 (40.0 %)	43 (62.3 %)	38 (51.4 %)	45 (59.2 %)	*0.021*
Progression of AAC (N/%)	17 (24.3 %)	36 (40.9 %)	30 (41.1 %)	37 (48.7 %)	*0.022*

Each quartile represents the number and percentage of individuals with prevalence and progression of AAC.

Table III. Prevalence and progression of abdominal aortic calcification based on the levels of GGT in quartiles separated by gender

	Serum levels of gamma glutamyl transferase (GGT) in IU/L					
		Q1 (< 16.25)	**Q2 (16.25-23.5)**	**Q3 (23.5-34)**	**Q4 (> 34)**	***p value***
Men	Prevalence of AAC (N/%)	24 (60.0 %)	24 (64.9 %)	21 (60.0 %)	27 (67.5 %)	0.918
	Progression of AAC (N/%)	13 (33.3 %)	18 (48.6 %)	14 (41.2 %)	23 (59.0 %)	0.134
		Q1 (< 11.75)	**Q2 (11.75-15)**	**Q3 (15-22.25)**	**Q4 (> 22.25)**	***p value***
Women	Prevalence of AAC (N/%)	14 (35.0 %)	18 (38.3 %)	15 (50.0 %)	19 (46.3 %)	0.537
	Progression of AAC (N/%)	10 (25.0 %)	16 (34.0 %)	10 (33.3 %)	16 (39.0 %)	0.603

Each quartile represents the number and percentage of individuals with prevalence and progression of AAC.

Table IV. Effect of elevated serum GGT levels on changes to bone mineral density (BMD) in the lumbar spine and femoral neck in both sexes

		Q1-3 (≤ 34)	**Q4 (> 34)**	***p* value**
Men	% Change to lumbar BMD	-1.43 ± 4.10	-0.27 ± 5.97	0.229
	% Change to femoral neck BMD	0.16 ± 4.40	-0.56 ± 4.12	0.108
		Q1 (≤ 22.25)	**Q4 (≤ 22.25)**	***p* value**
Women	% Change to lumbar BMD	1.23 ± 4.60	-1.34 ± 6.21	*0.012*
	% Change to femoral neck BMD	1.35 ± 4.64	-0.10 ± 3.78	0.090

DISCUSSION

The outcomes of this study reveal a clear association between serum GGT levels and abdominal aortic calcification in a cohort of men and women with a mean age of 68 years, as former studies on coronary aortic calcification had already confirmed (10-13). Gender-specific analysis proved that in both men and women, the highest GGT quartile was associated with more than a 3-fold increase in the progression of calcification, including new calcifications and the worsening severity of the already existing ones. Other authors have also noted that GGT is independently associated with coronary aortic calcification only in women, not in men (11). However, some authors found this association in both genders (10,13) or only in men (12). All these works come from cross-sectional, unlike our study involving longitudinal tracking of abdominal aortic calcification progression.

Atherosclerosis could be a precursor to vascular calcification, and the association between GGT and atherosclerosis is well-established. However, the exact mechanisms remain to be elucidated (15). Several mechanisms have been proposed though. The first mechanism suggests that GGT is associated with multiple atherosclerotic risk factors. Former studies demonstrated that serum GGT levels were associated with hypertension, metabolic syndrome, and diabetes *mellitus* (8,16-18), and increase insulin resistance (19). The second mechanism proposed is that GGT serves as a biomarker of oxidative stress. Physiologically, GGT acts as a protein catalyst in the degradation of glutathione, the body's primary thiol antioxidant, potentially making it a proatherogenic marker (20). The third mechanism proposed is subclinical chronic inflammation. Former studies showed that there was a relationship between GGT levels and the levels of C-reactive protein (CRP) (21,22). Inflammation is known to be a crucial mechanism in all stages of cardiovascular disease (23). GGT mediates the interconversion of the inflammatory mediator leukotriene C4 containing glutathione into leukotriene D4 (24). The fourth mechanism proposed is the straightforward atherogenic potential

of GGT. Some studies have identified enzymatically active GGT in coronary and carotid atheromas at the time of surgical atherectomy (25,26). It has been suggested that GGT may participate in disease progression by modulating one or more redox-sensitive processes involved in atherosclerosis (20,25).

Not only the association between serum GGT levels and coronary aortic calcification has been established, but also several studies have also associated serum GGT levels to valvular calcification (27,28). The role of GGT in tissue calcium balance was already studied by Niida et al. (29) who proved that the overexpression of GGT in transgenic mice accelerated bone resorption, leading to osteoporosis, possibly by stimulating the receptor activator of nuclear factor kappa-B ligand (RANKL) (30). In our study, higher GGT values in women were associated with greater bone loss at lumbar spine level, with an obvious although non-significant trend reported at femoral neck level. Perhaps focusing on aortic calcifications in the vicinity of the spine contributed to the lack of a relationship with another bone segment like the hip assuming that the theory of greater bone demineralization leading to greater calcification is correct (1). Femoral calcification should have been determined here to see if it was associated with changes to femoral BMD. In men, no effects were seen, probably due to women's greater tendency towards osteoporosis.

This study has several limitations. Firstly, the serum determination of GGT was only conducted in the second cross-sectional analysis, thus limiting the associations found. Secondly, another potential limitation is that aortic calcification might overlap or juxtapose with lumbar vertebrae, leading to increased BMD values not due to actual changes to bone mass. However, this fact would not limit but rather reinforce our results as the overlay of calcifications might increase but not decrease BMD, thus reducing the real association between vascular calcification progression and BMD decrease like some studies suggest (1). Furthermore, the evaluation of vascular calcification was conducted on a plain x-ray and more sensitive imaging modalities were never used. It is also possible that some of the individuals who attended the second follow-up at 4 years would've done so due to worse physical condition compared to those who didn't attend, although no clear selection biases were ever reported (31).

Despite these limitations, the study also possesses significant strengths, such as the substantial response rate of participants both at baseline (50 %) (32) and at the 4-year follow-up (70 %). The degree of inter-observer reproducibility to assess vascular calcification supports its use as a diagnostic criterion. Finally, unlike other studies, this one was prospective rather than cross-sectional, as most referenced studies are. This reinforces the validity of the findings, as well as their stronger degree of association.

In conclusion, we can assert that elevated GGT levels were associated with the presence and progression of abdominal aortic calcification, and in women, with greater lumbar BMD loss. When analyzed by gender, higher GGT values were associated with the progression of abdominal aortic calcification, thus confirming the utility of this marker as a cardiovascular risk factor regardless of gender and alcohol consumption.

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Original

Should the FRAX tool include other variables to assess fragility-related osteoporotic fractures?

Carlos Gómez Alonso[1], Minerva Rodríguez García[2,3], Teresa Naves López[1], Mónica Llaneza Faedo[1], Carmen Palomo Antequera[4], Laura Naves Mendívil[1], José Luis Fernández Martín[1,3], Manuel Naves Díaz[1,3]

[1]Bone Metabolism Clinical Management Unit. Hospital Universitario Central de Asturias. Universidad de Oviedo. Institute of Health Research of the Principality of Asturias (ISPA). Oviedo, Spain. [2]Nephrology Clinical Management Area. Hospital Universitario Central de Asturias. Universidad de Oviedo. Institute of Health Research of the Principality of Asturias (ISPA). Oviedo, Spain. 3REDinREN, Carlos III Health Institute (ISCIII). Madrid, Spain. [4]Internal Medicine Clinical Management Unit. Hospital Universitario Central de Asturias. Universidad de Oviedo. ISPA. Oviedo, Spain

Abstract

Introduction and objective: the objective of this study was to assess the significance of variables not included in the FRAX tool regarding the incidence of osteoporotic fractures.

Materials and methods: a total of 316 women older than 50 years were followed for 8 years. The variables collected (age, BMI, previous fracture, parental history of hip fracture, smoking habit, use of glucocorticoids, femoral neck BMD) and those not collected by the FRAX tool (age at menarche, menopause, fertile years, nulliparity) were studied.

Results: age and parental history of hip fracture were associated with hip fractures, but so were age at menopause and fertile years. Age [odds ratio (OR), 1.09; 95 % confidence interval (CI), 1.01-1.17] and age at menopause [OR, 0.90; 95 %CI, 0.82-0.99] were associated with hip fractures after the multivariate analysis adjusted for age and BMI. BMI, femoral neck BMD and nulliparity were associated with the occurrence of Colles fractures. According to the multivariate analysis, only nulliparity was associated with Colles fractures [OR, 4.59; 95 %CI, 1.59-13.26)]. Major osteoporotic fractures were significantly associated with parental history of hip fracture, nulliparity, and fertile years. According to the multivariate analysis, the parental history of hip fracture [OR, 3.26; 95 %CI, 1.23-8.61], nulliparity [OR, 3.07; 95 %CI, 1.48-6.37], and fertile years [OR, 0.92; 95 %CI, 0.87-0.98] were associated with the occurrence of major osteoporotic fractures.

Conclusions: among the FRAX variables, age and parental history of hip fracture were associated with the incidence of major osteoporotic and hip fractures. However, the significance of other gynecological variables was similar, which is indicative that they should certainlay be taken into consideration during patient history assessment.

Keywords: FRAX. Major osteoporotic fracture. Hip fracture. Incidence. Gynecological variables.

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Correspondence:
Manuel Naves Díaz. Unidad de Gestión Clínica de Metabolismo Óseo. Hospital Universitario Central de Asturias. Avda. de Roma, s/n. 33011 Oviedo, Spain
e-mail: mnaves.huca@gmail.com

INTRODUCTION

Despite the availability of a wide therapeutic arsenal and bone densitometry, many patients at risk of fragility-related osteoporotic fractures remain undiagnosed and untreated. Back in 2019, it was estimated that 2,985,000 individuals in our country suffered from osteoporosis (79 % of them, women). Only 36 % of the 1,827,000 women diagnosed with osteoporosis receive treatment, underscoring a treatment gap of 64 % (1). It is estimated that up to 80 % of individuals who have experienced, at one time or another at least, 1 fragility fracture lack proper diagnosis, have not been identified correctly, and consequently, do not receive the necessary diagnosis or subsequent proper management.

The concern surrounding the clinical management of osteoporosis has stimulated the development of procedures to assess the risk of fracture using key risk factors. A group of experts developed a tool called FRAX to identify individuals at higher risk of sustaining fractures within the next 10 years, by combining key factors of risk of fracture and adding bone mineral density (BMD) when available (2). Among fracture-related factors, decreased BMD has been identified as the primary risk factor because of its close relationship with bone strength. Other factors contributing significantly to the risk of fracture have also been identified including family and personal history of fragility fractures, low body weight, smoking, and age, which is known to be one of the most important independent predictors of fracture other than BMD.

However, we should admit that FRAX is not a perfect tool and has been criticized since its inception for not including certain risk factors widely discussed in the medical literature available, such as spine BMD, bone markers, use of benzodiazepines, or history of risk of fall (3-7). Therefore, the objective of this study was to assess if the variables not included in this algorithm, particularly certain gynecological variables, should be included based on the role they play on fragility fractures.

MATERIAL AND METHODS

A total of 316 women older than 50 years were randomized from the Oviedo municipal registry in Oviedo, Asturias, Spain. These women had previously participated in the EVOS study, which was initially designed to determine the prevalence of vertebral fractures across Europe. Also, they were asked to fill out a specifically designed survey for the EVOS study, which exhibited good reproducibility (8,9). This survey included questions on clinical variables such as weight and height to calculate the body mass index (BMI), osteoporosis-related risk factors such as previous fractures, parental history of hip fractures, smoking habits, and use of glucocorticoids for over 3 months. Additionally, other variables were analyzed, such as age at menarche, age at menopause, fertile years, and nulliparity. The femoral neck BMD of this cohort was also established.

This cohort was prospectively followed for 8 years through 4 postal surveys to establish the incidence of non-vertebral osteoporotic fractures during this period. All osteoporotic fractures (hip, Colles, humerus, rib, pelvis, and tibia) excluding skull and limb fractures were confirmed through x-rays, or medical reports. The total number of participants in the final follow-up was 223, with a participation rate of 81.3 % at the 8-year follow-up (excluding deaths). The participation rates for the 3 previous postal follow-ups were 87.1 %, 87.5 %, and 82.4 %, respectively.

DENSITOMETRIC ANALYSIS

The femoral neck BMD was measured using a Hologic® QDR-1000 DXA densitometer (Hologic Inc., Waltham, MA, United States). The coefficient of variation (CV) was 1.9 % (10). Precision and quality control were kept through daily scans using a lumbar spine phantom, resulting in a CV of 0.0 ± 0.1 %.

All studies conducted followed the principles established in the Declaration of Helsinki and received formal approval from the Regional Clinical Research Ethics Committee of the Principality of Asturias, Spain.

STATISTICAL ANALYSIS

Data analysis was conducted using SPSS version 25.0 for Windows. Quantitative variables were analyzed using the Student t test, and the qualitative ones using the chi-square test.

To conduct a multivariate analysis on the impact of multiples risk factors, included or not in FRAX calculations, on the incidence of non-vertebral osteoporotic fractures, logistic regression adjusted for age and BMI was used.

RESULTS

Table I illustrates the baseline clinical characteristics of the variables used by the FRAX tool, as well as other variables not included in this algorithm, such as age at menarche and menopause, fertile years, and nulliparity. The mean age was 65 years and the BMI, 28.6 kg/m^2. The femoral neck BMD (0.683 g/cm^2) fell within the range observed for the Spanish population aged 60-69 years

(0.694 g/cm^2). The parental history of hip fracture was reported by 7.7 % of participants, and the prevalence of previous non-traumatic fractures was 20 %. Less than 10 % (8.5 %) had used corticosteroids, and 4.7 % of women were smokers. Regarding the gynecological and obstetric variables, notable figures include a mean menarche, menopause, and fertile age of 14, 48.8, and 34.8 years, respectively. Approximately 17.5 % of women had never been pregnant (nulliparity).

Table I. Description of the baseline clinical characteristics of the study population

Clinical characteristics	Study women (*n* = 316)
Age (years)	65.3 ± 9.0
BMI (kg/m^2)	28.6 ± 4.3
Femoral neck BMD (g/cm^2)	0.683 ± 0.108
Parental hip fracture history (N, [%])	24 (7.7 %)
Previous non-traumatic fracture (N, [%])	64 (20.4 %)
Use of glucocorticoids (N, [%])	27 (8.5 %)
Current smoking (N, [%])	15 (4.7 %)
Age at menarche (years)	14.0 ± 2.0
Age at menopause (years)	48.8 ± 4.9
Fertile years (years)	34.8 ± 5.2
Nulliparity (N, [%])	55 (17.5 %)

The univariate analysis showed that, among the variables used to build the FRAX algorithm, only age and parental history of hip fracture were actually associated significantly with the incidence of hip fracture. However, age at menopause and fertile years were also associated (Table II). We should mention that, among fractured women, no use of corticosteroids or smoking habits were reported. The multivariate analysis adjusted for age and BMI only showed significant associations between age [OR, 1.09; 95 % confidence interval (CI), 1.01-1.17] and age at menopause [OR, 0.90; 95 %CI, 0.82–0.99] with incidental hip fractures.

In the univariate analysis, BMI, femoral neck BMD, and nulliparity were associated with the occurrence of incidental Colles fractures (Table III). According to the multivariate analysis, only nulliparity was associated significantly with the occurrence of Colles fractures [OR, 4.59; 95 %CI, 1.59-13.26)].

Regarding the incidence of major osteoporotic fractures, the univariate analysis showed significant associations with the parental history of hip fracture, nulliparity, and fertile years (Table IV). According to the multivariate analysis, the parental history of hip fracture [OR, 3.26, 95 %CI, 1.23-8.61)], nulliparity [OR, 3.07, 95 %CI, 1.48-6.37)], and fertile years [OR, 0.92, 95 %CI, 0.87-0.98)] were associated with the occurrence of incidental major osteoporotic fractures.

Table II. Association between clinical variables and the presence or absence of incidental hip fractures

Incidental hip fractures		Yes (*n* = 11)	No (*n* = 305)	*p* value
Variables included in FRAX	Age (years)	*72.7 ± 6.9*	*65.0 ± 8.9*	*0.005*
	BMI (kg/m^2)	26.7 ± 4.4	28.6 ± 4.3	0.143
	Femoral neck BMD (g/cm^2)	0.632 ± 0.062	0.684 ± 0.108	0.174
	History of parental hip fracture (N, [%])	*3 (27.3 %)*	*21 (7.0 %)*	*0.044*
	Previous non-traumatic fracture (N, [%])	4 (36.4 %)	60 (19.9 %)	0.245
	Use of glucocorticoids (N, [%])	0 (0.0 %)	27 (8.9 %)	0.752
	Current smoking (N, [%])	0 (0.0 %)	15 (4.9 %)	0.752
Variables not included in FRAX	Age at menarche (years)	14.0 ± 1.7	14.0 ± 2.0	0.996
	Age at menopause (years)	*45.3 ± 4.6*	*48.9 ± 4.9*	*0.015*
	Fertile years (years)	*31.3 ± 4.6*	*34.9 ± 5.2*	*0.022*
	Nulliparity (N, [%])	3 (27.3 %)	52 (17.1 %)	0.414

Italicized values indicate significant differences between the two groups.

Table III. Association between clinical variables and the presence or absence of incidental Colles fractures

Incidental Colles fractures		Yes (*n* = 11)	No (*n* = 305)	*p* value
Variables included in FRAX	Age (years)	65.0 ± 7.3	65.3 ± 9.0	0.910
	BMI (kg/m^2)	*26.2 ± 3.1*	*28.7 ± 4.3*	*0.031*
	Femoral neck BMD (g/cm^2)	*0.627 ± 0.045*	*0.685 ± 0.109*	*0.008*
	History of parental hip fracture (N, [%])	3 (27.3 %)	21 (7.0 %)	0.082
	Previous non-traumatic fracture (N, [%])	3 (25.0 %)	61 (20.5 %)	0.965
	Use of glucocorticoids (N, [%])	2 (13.3 %)	25 (8.3 %)	0.373
	Current smoking (N, [%])	0 (0.0 %)	15 (5.0 %)	0.474
Variables not included in FRAX	Age at menarche (years)	14.3 ± 2.5	14.0 ± 2.0	0.602
	Age at menopause (years)	47.6 ± 6.9	48.8 ± 4.8	0.353
	Fertile years (years)	33.0 ± 7.7	34.9 ± 5.1	0.389
	Nulliparity (N, [%])	*7 (46.7 %)*	*48 (16.0 %)*	*0.002*

Italicized values indicate significant differences between the two groups.

Table IV. Association between clinical variables and the presence or absence of incidental major osteoporotic fracture

Incidental major osteoporotic fractures				
Variables included in FRAX	Age (years)	67.2 ± 8.7	65.1 ± 9.0	0.175
	BMI (kg/m^2)	27.7 ± 3.8	28.7 ± 4.3	0.188
	Femoral neck BMD (g/cm^2)	0.653 ± 0.074	0.686 ± 0.110	0.147
	History of parental hip fracture (N, [%])	*6 (16.7 %)*	*18 (6.5 %)*	*0.025*
	Previous non-traumatic fracture (N, [%])	9 (25.0 %)	55 (19.9 %)	0.472
	Use of glucocorticoids (N, [%])	2 (5.6 %)	25 (8.9 %)	0.752
	Current smoking (N, [%])	1 (2.8 %)	14 (5.0 %)	0.472
Variables not included in FRAX	Age at menarche (years)	14.6 ± 2.2	13.9 ± 2.0	0.075
	Age at menopause (years)	47.5 ± 5.7	49.0 ± 4.8	0.091
	Fertile years (years)	*32.6 ± 6.0*	*35.1 ± 5.0*	*0.009*
	Nulliparity (N, [%])	*13 (36.1 %)*	*42 (15.1 %)*	*0.002*

Italicized values indicate significant differences between the two groups.

DISCUSSION

The results from this study illustrate the significance of some gynecological variables not included in the FRAX algorithm that could carry specific weight. We should mention the significant protective effect of delaying menopause age on the incidence of hip fractures, or the protective effect of fertile years or pregnancy on major osteoporotic fractures.

Undoubtedly, FRAX is a valuable tool to aid clinicians in the identification of individuals needing osteoporosis treatment and those who do not. Also, it is the only algorithm validated across various population cohorts, with or without BMD data, and available in multiple languages. It can equally serve as an inclusion criterion in clinical trial design where fracture is an endpoint. However, it does have well-recognized limitations (11).

Within these limitations, particularly pertinent to this study, is that FRAX has not been validated in Spain (12). Also, FRAX does not include variables that could be challenging for general practitioners to obtain, such as physical activity measurements, vitamin D deficiency, bone turnover markers, or bone loss. Another variable not included is falls despite being a recognized risk factor for non-vertebral fractures, especially hip fractures. Falls are excluded mainly due to the lack of standardized assessment methods. Perhaps this is why gynecological variables were never added to the FRAX algorithm, despite having a specific weight on the occurrence of fragility-related osteoporotic fractures (13). Also, it is possible that the confinement of these variables to women alone may require 2 separate calculators for each gender, which could be operationally cumbersome.

A study of our geographical context showed that not all risk factors included in the FRAX algorithm were significant when fractured and non-fractured women were compared (14). Specifically, age, previous fracture, and baseline osteoporosis were significant. However, excessive alcohol consumption, use of glucocorticoids, rheumatoid arthritis, low BMI, and parental hip fracture history did not show any significance, despite the latter 2 being considered strong or very strong risk factors in FRAX (14). In our case, BMI was associated only with the occurrence of osteoporotic Colles fractures, an association that wasn't found in the multivariate analysis. Similarly, the history of parental hip fracture was associated with the occurrence of hip and major osteoporotic fractures in the univariate models, but only remained significant with major osteoporotic fractures at the multivariate level.

Some authors suggest that the FRAX tool should add gynecological variables, such as menopause, including its onset and duration (15). The findings from our study are not intended to challenge the utility of FRAX. We view FRAX as a highly useful tool to assess individuals at high risk of sustaining fragility fractures. However, clinicians should consider these gynecological variables during the clinical examination of women older than 50 years, in addition to using the FRAX tool, because these variables have shown an association with the incidence of osteoporotic fractures (13,16-20). In our study, regarding hip fractures, delaying menopause by 1 year reduced its incidence by 10 %, which is similar to the 9 % increased risk associated with 1 additional year of aging. Similarly, for major osteoporotic fractures, the parental history of hip fracture was associated with a 3-fold higher risk, which is somewhat similar to the risk associated with nulliparity. Each fertile year cut the risk of major osteoporotic fracture by 8 %.

Our study has several limitations. The survey used to collect osteoporosis risk factor data was interviewer-administered rather than self-administered, which potentially introduces response biases. Some FRAX variables, such as rheumatoid arthritis, secondary osteoporosis, or alcohol consumption, could not be assessed due to the survey design. Another possible, yet inevitable, limitation is the time elapsed between gynecological changes and the study data collection. The study's limited number of incidental osteoporotic fractures and the relatively small cohort size (n = 316) could also be considered limitations.

Despite these limitations, we believe the study has significant strengths. On the one hand, the cohort analyzed participated in the EVOS-EPOS study, being our center one of the 5 centers that completed all the study guidelines. Additionally, the response rate across the 4 postal follow-ups over 8 years exceeded 80 %, strongly supporting the sample's representativeness (21).

In conclusion, some gynecological variables like age at menopause, fertile years, and nulliparity should be considered risk factors for the occurrence of fragility fractures in the patients' medical records. Future studies should clarify whether adding these gynecological variables alongside the FRAX algorithm could enhance the predictive capabilities of this tool widely used in the routine clinical practice.

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Original

Efficacy of an oral collagen therapy compared with intra-articular therapies (hyaluronic acid and platelet-rich plasma) to treat knee osteoarthritis

Eduardo Álvarez Lozano[1], Alejandro González Parás[1], Ramón Quintanilla Loredo[1], Margia Victoria Cerda García[1], Francisco Forriol[2], Beatriz Bravo Molina[2]

[1]Orthopaedic Surgery Department. Hospital Eleuterio González. School of Medicine. Universidad Autónoma de Nuevo León. Monterrey, México.
[2]School of Medicine. Universidad CEU-San Pablo. Campus de Montepríncipe. Madrid, Spain

Abstract

Introduction: osteoarthritis is a chronic and progressive disease. It affects over 30 % of people older than 60. Osteoarthritis is currently recognized as a multifactorial disease. Various conservative treatments are used in the management of knee osteoarthritis (NSAIDs, analgesics, and intra-articular therapy). We conducted a randomized clinical trial to determine if a 10 g therapy of hydrolyzed collagen along with 100 mg fucoidan (Hydroidan pro, Acten, Switzerland) is more effective than intra-articular therapies.

Methods: we divided patients into 3 groups. The first group received 23 g of ACTEN®, daily, for 3 months. The other groups received a single intra-articular injection of hyaluronic acid (5 ml) or platelet-rich plasma (3 ml). We used the WOMAC scale, the SF-12 scale, and the VAS for pain at baseline, and 4, 12, and 24 weeks later.

Results: we enrolled 108 patients with grade II-III knee osteoarthritis who underwent a 24-week follow-up study. The mean age was 57 years (53-65). The three groups showed low scores in the WOMAC group ($p < 0.001$). The collagen with fucoidan group had lower WOMAC and VAS scores compared with the hyaluronic acid and platelet-rich plasma groups at 24 weeks ($p < 0.001$).

Conclusions: collagen along with fucoidan taken orally, daily, for 12 weeks seem to have better results in the WOMAC and VAS scales compared with intra-articular therapies such as hyaluronic acid or platelet-rich plasma. Combined oral and intra-articular therapies should be tried to determine their efficacy profile.

Keywords:
Knee.
Osteoarthritis.
Cartilage.
Fucoidan.
Hyaluronic acid.
Platelet-rich plasma.

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Conflicts of interest: the authors declare no conflict of interest.

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Correspondence:
Beatriz Bravo Molina. School of Medicine. Universidad CEU-San Pablo. Campus de Montepríncipe. 28668 Alcorcón. Madrid, Spain
e-mail: beatriz.bravomolina@ceu.es

INTRODUCTION

Osteoarthritis (OA) is currently recognized as a multifactorial disease in which various factors can generate and perpetuate damage to articular cartilage, with the subsequent response of the synovial membrane and subchondral bone. Knee OA has been regarded as a purely mechanical condition, with the emphasis on joint overloads associated with axis changes, traumatic injuries, and multi-ligament instabilities. When chondral extracellular matrix (ECM) is compromised, there is less water retention and the tissue loses resistance, resilience, and elasticity to compression, thereby increasing damage to the surrounding tissue. Due to the low rate of cell turnover and poor reparative capacity, the cartilage fails to compensate for the damage sustained (1,2). Regardless of the original cause of the damage, synovial membrane fibroblasts respond by secreting various cytokines and inflammatory factors (IL-1, TNF-α, TGF-β, IL-8, among others) (3,4). These inflammatory factors remain present in the joint, regardless of the corrective treatment of the original cause of chondral damage (ligament stabilization, fracture reduction, axe correction, etc.), being able to maintain the progression of the damage (1,2,5).

Currently, various conservative treatments are used to treat knee osteoarthrosis, including drug therapy with non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics (AAS, paracetamol). Also, natural products, like glycosaminoglycans, chondroitin-sulfate, or collagen are advised. Intra-articular therapy involves the restoration of the usual biological properties, viscosity and elasticity, and synovial fluid using hyaluronic acid, which regulates various cellular activities and restores the properties of synovial fluid. On the other hand, platelet-rich plasma (PRP), that uses a high concentration of platelets (2 to 4 times higher), releases growth factors with chondrogenic properties and anti-inflammatory cytokines.

The objective of this study is to determine if an oral therapy based on 10 g of hydrolyzed collagen along with 100 mg fucoidan (Hydroidan Pro, Acten, Switzerland)—a sulfated polysaccharide that comes from some types of brown algae and has proven to help reduce inflammatory factors—effectively reduces symptoms of grade II-III gonarthrosis on the Kellgren and Lawrence scale compared with intra-articular hyaluronic acid, or intra-articular platelet-rich plasma.

MATERIALS AND METHODS

STUDY DESIGN AND ETHICAL ASPECTS

This was a prospective, longitudinal, analytical, randomized, and single-blind study to assess the efficacy profile of an oral therapy (10 g hydrolyzed collagen along with 100 mg fucoidan, Hydroidan Pro, Acten, Switzerland) compared with intra-articular treatments (hyaluronic acid and platelet-rich plasma) to treat knee osteoarthritis. A digital app for randomization (https://www.randomizer.com) was used to allocate patients to 3 different groups. Following the center review board approval (OR17-00016) patients were actively recruited in the Department of Orthopedics and Traumatology. All patients provided their prior written informed consent to participate in the study. This study was conducted in accordance with the World Medical Association Declaration of Helsinki. The resources and funding to conduct this study were provided by our hospital Department of Orthopedics and Traumatology.

PARTICIPANTS AND STUDY SUBJECTS

From October 2017 through November 2019, we invited all patients aged between 40 and 90 years with diagnosed knee osteoarthritis (based on the American College of Rheumatology criteria), a > 12-month history of symptoms, and grade II-III osteoarthritis in the Kellgren-Lawrence classification to join our study. Exclusion criteria included pregnant or breastfeeding woman, rheumatoid arthritis, knee surgery or arthroscopy, use of intra-articular steroids, hyaluronic acid, or platelet-rich plasma in the previous 9 months, cancer in the past 5 years, glucosamine and chondroitin therapy in the previous 6 months, smokers (20 or more packs of cigarettes/year), alcohol users (50 or more grams/week), comorbidities such as gout (uric acid of 6.8 mg/dL or more), chronic renal disease (GFR < 60 mL/min/1.73 m^2), non-controlled diabetes mellitus (Hb1Ac > 7 %), non-controlled hypertension (> 120/> 80 or more), or patients who were participating in different studies. Exclusion criteria included a follow-up or missing a dose of the oral treatment.

During recruitment period from October 2017 through November 2019, a total of 301 patients were scheduled to evaluate the clinical and radiographic criteria. However, 190 patients were ineligible (78 patients, Hb1Ac > 7; 34 patients, BP > 120/> 80; 32 patients: Kellgren-Lawrence grade I or IV; 31 patients on steroid therapy 9 months prior; 10 patients on glucosamine or chondroitin therapy 6 months prior; 5 patients with a history of cancer in the past 5 years); and 3 patients refused to participate in the study. A total of 108 patients were included in the study in 3 groups: group 1: n = 36, group 2: n = 36, and group 3: n = 36. No patients were lost or excluded at the follow up.

No differences were observed for any demographic or clinical outcome variable.

The baseline characteristics included age, biological sex, height, weight, BMI, Kellgren-Lawrence grade and knee. The baseline score of the three groups showed no statistically significant differences. The mean age was 57 (53-65) years. Twenty-six patients (24.1 %) were men and eighty-two (75.9 %) were women. The mean body

mass index was 30.9 ± 5.4 kg/m^2, being most patients ranked as grade I obesity. A total of 46 patients (42.6 %) were grade II according to the Kellgren-Lawrence scale while 62 (57.4 %) were grade III. A total of 45 (41.7 %) had more pain in their right knee and 63 (58.3 %) in their left knee (Table I and Fig. 1).

This study proposed a 1:1:1: randomization into the 3 groups. A correlative identification number was given after the informed consent was signed. The patients enrolled were assigned to one of three groups (group 1, collagen-fucoidan; group 2, hyaluronic acid, and group 3, platelet-rich plasma). Patients were randomized by a staff member from our hospital who wasn't engaged in this study.

Group 1 (hydrolyzed collagen plus 100 mg of fucoidan, Hydroidan Pro, Acten, Switzerland) received a single dose of a saline solution (5 mL) as placebo, and a 23 g dose of Hydroidan Pro orally, daily, for 24 weeks. Group 2 (hyaluronic acid) received a single dose of hyaluronic acid (5 mL) and a 23 g dose of chlorophyll as placebo. Group 3 (platelet-rich plasma) received a single dose of platelet-rich plasma (3 mL) and a 23 g dose of chlorophyll as placebo. Collagen and chlorophyll were changed to a metal-like plastic bag and the syringes of saline solution and hyaluronic acid or platelet-rich plasma were personally delivered to the doctor working on the knee infiltrations.

Table I. Demographic aspects of the patients

Sex (n [%])	
Men	26 (24.1 %)
Women	82 (75.9 %)
Age	57 (53-65)
BMI n (SD)	30.9 ± 5.4
Kellgren-Lawrance scale (n [%])	
II	46 (42.6 %)
III	62 (57.4 %)
Knee affected (n [%])	
Right	45 (41.7 %)
Left	63 (58.3 %)

Infiltration technique

All infiltrations were performed by the same physician. Patients laid down in prone position. Asepsis and antisepsis were achieved with povidone iodine 8 %, then a sterile field was used to delimitate the working area. Patients were infiltrated with 3 mL of lidocaine (20 mg/mL) for anesthesia. The external suprapatellar technique was used in 102 patients (96.2 %) and the external subrotulian technique in 4 (3.7 %), which was left to the physician's criterion. After infiltration, the area was covered with a band-aid and the knee was flexed. Prophylactic antibiotics were not administered.

OUTCOME MEASUREMENTS

Demographic characteristics (age, biological sex, weight, height, and BMI), Kellgren-Lawrence classification, affected knee and comorbidities were addressed and col-

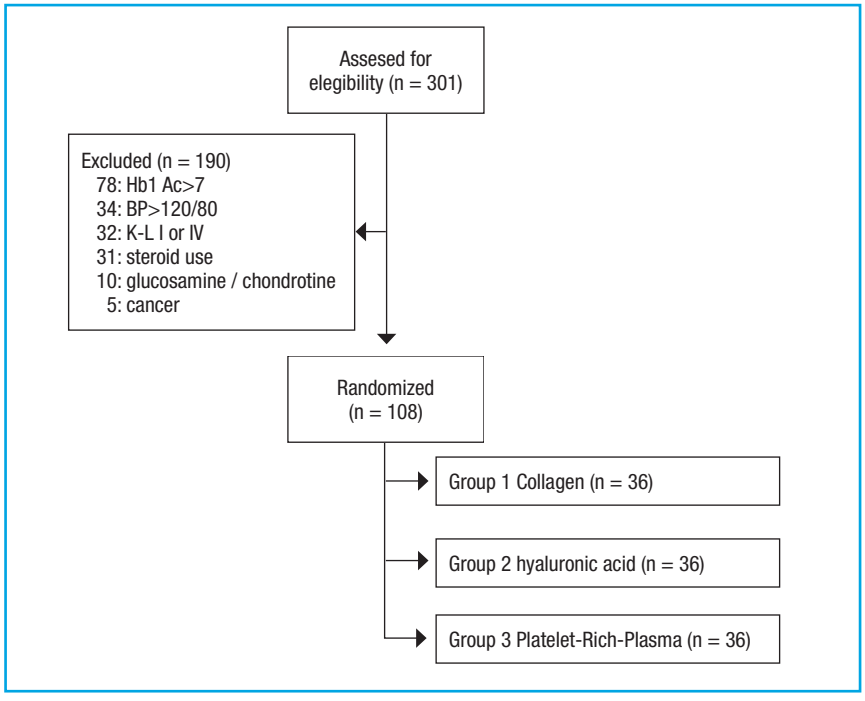

Figure 1. Flowchart of the patients' randomization.

lected. We used the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), the SF-12, and the VAS scale at baseline, and 4, 12 and 24 weeks later. The primary endpoint was the WOMAC scale at the 24-week follow-up compared to baseline. The secondary endpoints were the VAS and the SF-12 scale at the 24-week follow-up up compared to baseline. The WOMAC scale used was the 3.1 version (0-96). The SF-12 scale was the 2 version one (both bphysical and mental). The VAS scale was the linear one (0-10).

STATISTICAL ANALYSIS

The study sample size was calculated using an adjusted mean estimation formula in two populations, with an expected decrease of 60 ± 15 points for WOMAC in group 1 (Hydroidan Pro) vs 50 ± 10 points in the remaining therapies, with an 80 % statistical power and a two-tailed significance level of5 %. At least, a total of 29 patients were required per treatment group.

Statistical analysis was performed using IBM SPSS version 25 statistical package (Armonk, NY; IBM Corp.). The main characteristics of the population were described. Categorical variables were expressed as frequencies and percentages. The continuous ones as means ± standard deviation (SD) or median (interquartile range), after assessing the normality of data distribution using the Kolmogórov-Smirnov test. The intra-group comparisons of the different scores obtained throughout the different evaluations were performed using the Wilcoxon test. For intergroup comparisons, the deltas (Δ) of the differences of each measurement with respect to the baseline scores were calculated and compared using the Kruskal-Wallis's test. *Post hoc* analysis with Bonferroni correction was used to identify significant measurement or inter-group differences over time. p values < 0.05 were considered as statistically significant.

RESULTS

A comparison of the WOMAC score (at baseline, and 4, 12 and 24 weeks later) was made. In the three groups (Hydroidan Pro, HA ,and PRP) lower scores were seen at the 24-week follow-up, with a mean reduction from 84 down to 15 points in group 1 (Hydroidan Pro) ($p < 0.001$), 86 down to 41 points in group 2 (HA) ($p < 0.001$), and 84.5 down to 38.5 in group 3 (PRP) ($p < 0.001$) (Table II). After a *post-hoc* analysis, we found score differences on weeks 4, 12 and 24 compared with the baseline evaluation of the 3 lines ($p < 0.001$).

Compared with the level of pain reported by the visual analogue scale, we found a significant reduction in the 3 groups, with a mean 8 to 1 points in group 1 (Hydroidan Pro) ($p < 0.001$), 8 to 2 in group 2 (HA) ($p < 0.001$) and 8 to 1.5 in group 3 (PRP) ($p < 0.001$) (Table III). After the *post-hoc* analysis, pain reduction was significantly less in each of the evaluations (on weeks 4, 12, and 24) compared with the baseline score ($p < 0.05$).

After the Δ of change in the WOMAC scale on the score's calculation on weeks 4, 12 and 24 weeks compared with baseline, we found a reduction of 23.5, 15.5, and 15 points on week 4; 50, 32.5, and 31 on week 12; and 68, 46, and 46.5 on week 24 in all groups (Hydroidan Pro, HA and PRP, respectively) ($p < 0.001$). Additionally, the Hydroidan Pro group showed minor WOMAC scores compared with patients treated with hyaluronic acid and platelet-rich plasma ($p < 0.001$) (Fig. 2 and Table IV).

The lower pain reported with the VAS at the follow-up was significantly different among the groups, with lower scores being reported in patients treated with Hydroidan Pro compared with those treated with HA on weeks 4, 12, and 24 ($p = 0.016$, $p < 0.002$, and $p < 0.001$, respectively) and those treated with PRP on week 12 ($p = 0.0031$).

Table II. WOMAC scores of the 3 groups in the different weeks at the follow-up

Group	Baseline	4 weeks	12 weeks	24 weeks	*p*
Collagen-Fucoidan	84 (82-88)	59.5 (57.2-64.7)	37 (33-39)	15 (12-18)	< 0.001
Hyaluronic acid	86 (82.2-89)	68.5 (64-72)	53 (50-57)	41 (39-43)	< 0.001
Platelet-rich plasma	84.5 (82-89)	70 (67.2-73)	54 (51.2-57.7)	38.5 (34-42)	< 0.001

Table III. VAS score of the 3 groups in the different weeks at the follow-up

Group	Baseline	4 weeks	12 weeks	24 weeks	*p*
Collagen-Fucoidan	7 (5-8)	5 (4-7)	4 (2-6)	3 (2-5)	< 0.001
Hyaluronic acid	8 (6-9)	5 (4-7.7)	5 (3.5-8)	5 (4-7)	< 0.001
Platelet-rich plasma	7 (6-9)	5 (4-7)	5 (3-6)	5 (3-6)	< 0.001

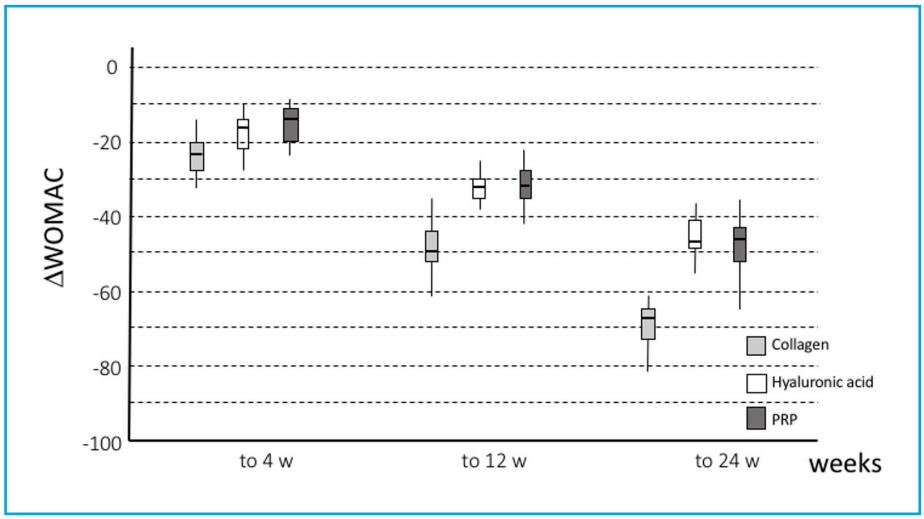

Figure 2. WOMAC scores of the 3 groups at the complete follow-up compared with baseline scores.

Table IV. WOMAC and VAS scores of the 3 groups at the 24-week follow-up compared with baseline values

	Collagen	HA	PRP	*p*
WOMAC	68 (65-72.7)	46 (41-47.7)	46.5 (43-52.5)	< 0.001
VAS	7 (6-8)	6 (4.2-7)	6 (5-8)	< 0.001

Also, we found lower scores in the PRP group compared with the HA group on week 12 ($p = 0.031$).

No adverse events occurred at the follow-up that were associated with the use of drugs. Three cases (2.7 %) of pain in the infiltration site for 3 or more days were reporte: 2 in the hyaluronic acid group and 1 in the Hydroidan Pro group.

DISCUSSION

The use of viscosupplementation in knee OA is extensive and fraught with heterogeneous trials with conflicting conclusions (6). There are two types of viscosupplementation hyaluronates and hylan. Hyaluronates are sodium hyaluronate and can be considered a drug as its mechanism of action is described mainly through a pharmacological mechanism that stimulates the endogenous synthesis of HA, which explains the extended duration of action. Hylan is considered an intra-articular implant since its mechanism of action is mainly through a physical mechanism. However, the mechanism of action of these products is not completely clear (7). By means of viscosupplementation, the production of IL-1 and other mediators of inflammation decreased. Likewise, the production of metalloproteases (MMP's) that degrade the articular cartilage decreased. In regard to the adverse events, although they have a good safety and tolerance profile, pain, swelling and effusion may occur in the infiltration area, known as temporary arthralgia, although some cases of pseudogout arthritis due to deposits of calcium pyrophosphate crystals have also been reported (7,8).

Within the biological treatment of osteoarthritis, PRP must be considered. PRP include a higher number of platelets than normal blood values. Platelets are enucleated cells traditionally characterized as main actors of the process of hemostasis, which is mediated by the release of proteins during its activation. PRP can be obtained and prepared from an individual's peripheral venous blood, through one or two subsequent centrifugation steps and with the use of basic laboratory materials or equipment (9). PRP is an effective intervention to treat knee OA without an increased risk of adverse events (10). The single administration of high volume pure PRP provided significant clinical benefit for 84.2 % of responders three months after the procedure. The KOOS total score significantly increased six months after the procedure, pain also significantly decreased and no difference was observed on MRI parameters (11). There are so many and such varied uses that detractors attribute this great variety of clinical applications and therapeutic benefits to a commercial benefit rather than a true effect on their "regenerative" capacity. The fact that platelets secrete growth factors and active metabolites leads us to believe that their use can have a positive influence in clinical cases that require rapid healing and tissue regeneration.

Advocates of this technique maintain that growth factors stimulate the synthesis of proteoglycans, aggrecans, and type II collagen by chondrocytes; induce synoviocyte proliferation, reduce catabolic effects of cytokines, such as interleukin-1 (IL-1) and MMP's, and the fact that it is an autologous preparation exempts it from harmful effects on joint tissues of patients (7,8). Among controversial issues regarding PRP, the lack of consensus on the exact composition stands out. Another frequent inconsistency is the composition of PRP and the heterogeneity of available techniques for its preparation. Several commercial presentations can vary significantly in the number of platelets, leukocytes, and erythrocytes. The initial centrifugation of the patient's blood separates red blood cells from plasma. Separated plasma may contain various concentrations of platelets with or without white blood cells. The platelets contained in this plasma can be further activated using thrombin, calcium chloride, calcium gluconate, freezing, and thawing, and this is subsequently applied to the area of injury as an infiltration while still liquid (12). This type of treatment can be conducted as a minimally invasive, outpatient treatment, providing a preparation directly to the area of injury with an immediate release of growth factors.

But for years the use of natural products that could "regenerate" cartilage has been defended. Among which are nutraceuticals, food products that have beneficial consequences for the body and can even act as drugs. Food products without any process or study must be distinguished from those found in nature or manipulated somehow. Collagen of animal origin is found in animal food and many collagen supplements are also sold. However, having them does not guarantee that it will be absorbed by the body. These supplements need to administered through a vehicle that allows its complete intestinal absorption so they can have an effect. In our case, collagen is added to a gel that allows the slow absorption of most of its collagen content. In addition, it is associated with another natural product, the extract of an algae, fucoidan, which is not a food, but has proven anti-inflammatory effects. Hence, there is a great variability of results in the different studies conducted, since not all collagens have the same quality or the same absorption capacity. As Deal and Moskowitz (13) put it, the name alone is not enough; you must know the doses, manufacturing and origin of the products that are indicated, because they're not all the same.

The intake of hydrolyzed collagen has been associated with pain relief and increased function in patients with OA. It has been suggested to use pharmaceutical grade hydrolyzed collagen as a modifying agent to treat OA based on the mechanisms of action of collagen as a tissue stimulant. Collagen as a nutritional supplement has been researched for the management of patients with OA and other types of joint pain. Experimental studies with bovine cartilage and cell cultures have shown that the administration of hydrolyzed collagen peptides increases type II collagen synthesis by chondrocytes (14). Moskowitz et al. (12) treated 52 patients of 56 years of age, with four treatments, three collagen preparations and egg albumin as control. During the study, patients were allowed to continue to use the analgesics or anti-inflammatory agents that they used to treat their symptoms before the study, maintaining a stable dose throughout their participation. All three collagen preparations were significantly superior to egg albumin in reducing pain compared to baseline. Side effects included mainly "an uncomfortable heaviness in the stomach". At the end of the test cycle with any of the collagen-containing preparations, analgesic consumption was significantly reduced compared to consumption before treatment, in contrast to the control group. No radiographic changes were seen during the study period. Lab test results indicated no changes in liver function studies or antibody titers in the 3 types of collagens studied (1). The authors suggested that collagen has a direct analgesic effect or that collagen administration provides a source of amino acids that act to improve matrix structure. Although this study describes an effect of collagen in the management of OA pain, factors such as variation in the degree of disease progression at the time of inclusion in the study, the inclusion of hips and knees as joints to be analyzed, the use of a not widely used outcome measurement scale, and a significant dropout rate represent caveats in the interpretation of research results (15).

Another study (16) administered daily 10 g of hydrolyzed collagen to over 100 patients from 1 to 6 months. Participants receiving the collagen had significantly higher plasma levels of hydroxyproline and a major component of collagen than those in the placebo group. Although these studies were open-label trials, which means that there is a limited level of scientific evidence, we can see the high degree of safety of use with a dose of 10 g/day of pharmaceutical grade hydrolyzed collagen.

Luo et al. (17) conducted a study in patients with knee osteoarthritis who received collagen, along with glucosamine and chondroitin sulfate in one group, with a control group who received placebo. At 12 weeks, the administration improved the experimental group regarding not only pain but also quality of life.

For Campbell et al. (18) intra-articular PRP is a viable treatment for knee OA and has the potential to lead to symptomatic relief for up to 12 months. There appears to be an increased risk of local adverse reactions after multiple PRP injections. Intra-articular PRP offers better symptomatic relief to patients with early knee degenerative changes. In the short-term follow-up (≤ 1 year), intra-articular PRP injection is more effective in terms of pain relief and function improvement to treat patients with knee OA than HA and placebo (17), and there is no difference in the risk of an adverse event between PRP and HA or placebo (20). Di Martino et al. (21) enrolled 167 patients with knee OA (Kell-

gren-Lawrence grade 0-3) randomized to undergo 3 blinded weekly intra-articular injections of either PRP or HA. Patients were prospectively assessed before treatment and for a median follow-up of 64 months. Both treatments effectively improved the knee functional status and symptoms over time up to 24 months. The PRP group still presents significantly higher values compared to baseline and HA though not statistically significant compared to baseline. A comparative analysis showed no significant intergroup difference in any of the clinical scores at any follow-up point. The median duration of patient subjective perception of symptomatic relief was 9 months for HA and 12 months for PRP. The latter did not provide an overall superior clinical improvement compared to HA in terms of symptomatic-functional improvement at different follow-up points or effect duration. Filardo et al. (22) included 192 patients with unilateral symptomatic knee with chronic pain or swelling with a Kellgren-Lawrence score of 0-3 on the X-rays. Patients underwent 3 weekly intra-articular injections of either PRP or HA. Both treatments proved effective improving the knee functional status and reducing symptoms with IKDC scores being obtained in both the PRP group and in the HA group. The comparative analysis of the 2 treatments showed no significant intergroup difference at any follow-up evaluation in any of the clinical scores used.

Hydroidan Pro is composed by 10 g of hydrolyzed collagen with 100 mg fucoidan. Fucoidan is a generic term for a class of molecules that are a class of polysaccharides composed of a main chain of fucose, fucopyranose, and natural sulfate, which are found in brown algae (echinoderms) and account for over 40 % of the dry weight of cell walls of algae. They have a wide spectrum of activity in biological systems. Its main function is to form a gel network to protect the floating structures of algae from drying out as they are exposed to air while their root and much of the stem are submerged in seawater (23). Fucoidan can be used in cosmetics, functional foods, dietary supplements, and in pet, livestock and aquaculture food supplements. To date, fucoidan has not been developed as a regulated therapeutic product yet. However, research on the use of fucoidan, specifically the one extracted from *Fucus vesiculosus*, has recently gained interest due to its biological activities and potential medical applications (24). Animal models of collagen-induced arthritis showed that orally administered fucoidan successfully inhibited pain (25). One of the physio-pathological components of great importance in the etiology of pain and inflammation in OA is the one associated with selective blocking of the migration and accumulation of neutrophils to the joint and the release of inflammatory mediators. This is related to its action on adhesion molecules found in platelets (P-selectins), and leukocytes (L-selectins), which theoretically prevents the passage of inflammatory cells to tissue spaces, attenuating inflammation. Carvalho et al. (23) demonstrated the effect of fucoidan to inhibit neutrophil infiltration and reduce the levels of pro-inflammatory cytokines and the symptoms of OA were inhibited by 52 % during 12 weeks of oral administration. There was no reduction of TNF-ß as a marker of inflammation. However, an accompanying study with healthy volunteers showed a reduction of IL-6, a marker of chronic inflammation. In an injection-induced arthritis model of zymosanÒ, a carbohydrate used to induce sterile inflammation experimentally, in knees of rats, the administration of a fucoidan preparation (*F. vesiculosus*) administered at intraperitoneal doses of 15 mg/kg, 30 mg/kg, and 50 mg/kg, 1 hour after the induction of joint inflammation reduced cell migration significantly. The dose of 30 mg/kg also improved the loss of glycose-minoglycans caused by zymosan®.

A small clinical trial (24) explored the use of a preparation of fucoidan (85 % w/w), *Macrocystis pyrifera* (10 % w/w) and *Laminaria japonica* (5 % w/w) in 12 subjects with OA randomized to take either 100 mg or 1000 mg capsules (75 mg or 750 mg fucoidan) orally for 12 weeks. There was a dose-response improvement in the participants. The lower dose reduced the average score by 18 %, while the higher dose improved the score by 52 %. They found also a reduction of leukotriene B4 and prostaglandin E2. The reduction of mediators of inflammation was associated with a reduction of joint pain. Predominant cells related to the inflammatory process induced in the ankle were granulocytes, not lymphocytes. The injection of fucoidan (10 mg/kg) reduced interactions between leukocytes and endothelial cells, and the number of cells decreased by nearly 60 % (26).

Intra-articular collagen injection with ChondroGrid (CG) a hydrolyzed (< 3 kDa) bovine collagen injectable formulation was injected in 70 patients affected by Kellgren Lawrence grade 1 to 4 knee OA and BMI < 30. These patients were given 3 CG injections and followed up for 6 months after the last administration. At the last follow-up, they showed a 50 % reduction of their median Lequesne score, a 50 % reduction of their VAS score at rest and in motion, and a ≥ 50 % reduction in all other scores under consideration (27). De Luca et al. (28) studied 20 patients with Kellgren Lawrence grade 1 to 4 knee OA who received three 4 mg/2mL injections of bovine hydrolyzed < 3 kDa type I collagen (ChondroGrid), 2 weeks apart. These patients were retrospectively assessed to compare scores collected before and 15, 45, and 225 days after the first injection. ChondroGrid induced type-II and inhibited type-I collagen deposition. Patients showed a 44 % Lequesne score and a 55 % VAS at moving score reduction.

Comblain et al. (29) demonstrated that a mixture of curcuminoids extract, hydrolyzed collagen, and green tea extract (COT) inhibited inflammatory and catabolic mediator's synthesis by osteoarthritic human chondrocytes. The compounds were more efficient inhibiting the interleukin-1β stimulated matrix MMP-3 expression than curcuminoids extract alone. In interleukin-1β-stimulated human chondrocytes, nitric oxide,

interleukin-6 and matrix MMP-3 productions reduced significantly with curcuminoids extract alone or together with hydrolyzed collagen and green tea extract (14). The COT mixture has beneficial effect on OA physiopathology by regulating the synthesis of key catabolic, inflammatory and angiogenesis factors.

Study limitations: the groups couldn't be completely blind because in one of them blood was drawn while in the other 2 it wasn't. It would be necessary to continue the study for a longer time to know if there are imaging changes. Ultrasonography or MRI could be useful for evaluation purpose. However, it complicates the control of these patients. On the other hand, they are not exclusive treatments, so the combination of intra-articular injections of HA or PRP along with oral treatment of hydrolyzed collagen should be tried.

Prolonged treatments with oral collagen, in combination or not with other therapies, can be useful in patients with OA to slow down the degenerative process with the occurrence of few adverse events. Few clinical scientific studies have been published to this date, so we recommend going deeper into their results to better understand their mechanism of action.

Hydrolyzed collagen along with fucoidan, taken orally daily for 12 weeks, seems to have better results in the WOMAC and VAS scales compared with intra-articular therapies such as hyaluronic acid or platelet-rich plasma. Combined oral and intra-articular therapies should be tried to determine the efficacy profile.

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Review

Cellular senescence as a pathogenic factor and potential therapeutic target in osteoporosis

Lorena Pena Larrea[1], Manuela de Blas Rodríguez[2], Manuel Naves Díaz[3], Carlos Gómez Alonso[3]

[1]Department of Traumatology and Orthopedic Surgery. Hospital Comarcal García Orcoyen. Estella, Navarra, Spain. [2]Clinical Management Unit of Bone Metabolism. Hospital Universitario Central de Asturias. Oviedo, Spain. [3]Clinical Management Unit of Bone Metabolism. Hospital Universitario Central de Asturias. Grupo de Metabolismo Óseo, Vascular y Enfermedades Inflamatorias Crónicas. Instituto de Investigación Sanitaria del Principado de Asturias (ISPA). RICORS 2040 - Enfermedad Renal del ISCIII. Oviedo, Spain

Abstract

Cellular senescence is a process induced by various types of stress that irreversibly cause cell cycle arrest and changes to the characteristics and functionality of cells, as well as the acquisition of a secretory phenotype that generates a pro-inflammatory environment. While, in certain contexts, it is beneficial for tissues and promotes organism development, senescence is a cellular fate implicated in the process of aging and age-related degenerative conditions. Senolytics are drugs that specifically eliminate senescent cells, and senomorphics are drugs that suppress their senescence-associated secretory phenotype (SASP) without inducing cell death. Therefore, therapeutic strategies targeting senescent cells (senolytics and senomorphics) as an underlying mechanism of aging emerge as an alternative with great potential to fight age-related diseases as a whole rather than individually. One of these conditions is osteoporosis where it has been experimentally described that drugs such as zoledronic acid have effects on preosteoblasts and act on senescent cells extending survival and opening up the possibility of treating age-related diseases with drugs already used in practice, which may have effects beyond the bone itself and increase overall survival. In this study, a review will be conducted in this rapidly growing field in recent years of undeniable translational interest.

Keywords:
Senescence. Osteoporosis. Senolytics. Senomorphics. Bone. Frailty. Senescence-associated secretory phenotype (SASP).

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Correspondence:
Lorena Pena Larrea. Department of Traumatology and Orthopedic Surgery. Secretary of Traumatology. Hospital Comarcal García Orcoyen. C/ Santa Soria, n.° 22. 31200 Estella, Navarra. Spain
e-mail: lorenapena89@gmail.com

INTRODUCTION

The remarkable increase in life expectancy since the mid-20th century has led to a rapid aging of the population (1). Globally, the population over 65 years old is growing at a faster pace compared to other population segments. As a matter of fact, it is estimated that by 2050, the overall number of octogenarians will triple the numbers from 2019 (2).

This increase in longevity, an achievement of improved life conditions and medicine itself, is accompanied by an increase in the burden of chronic diseases (cardiovascular, musculoskeletal, neoplastic, and neurological) (3-5), which not only has social and economic implications but also results in a loss of quality of life, functional limitations, frailty, and mortality (6,7). In the current context, developing strategies with a focus on treating aging as the common denominator of these conditions, rather than each specific disease, can be of tremendous relevance, extending health and delaying, preventing, or alleviating age-related disorders.

The cellular mechanisms involved in aging include telomere shortening, genomic instability, epigenetic alterations, mitochondrial dysfunction, loss of protein homeostasis (proteostasis), depletion and decline of stem cells, nutrient sensing deregulation, immune system decline, and cellular senescence (8,9). These processes are interrelated (10), linked and overlap. Senescence is not only a cause but also a consequence of several of them with senescent cells acting as true "synchronizers" of aging in different tissues, organs, or systems. Therefore, the objective of this work was to conduct a review in this rapidly growing field in recent years of undeniable translational interest.

CONCEPT

Cellular senescence is defined as a stable state of cell cycle arrest in response to different stimuli, in which cells cease proliferation and acquire an altered phenotype, thus losing their primary functionality. The concept was first described back in 1961 by Hayflick et al. (11) in human diploid fibroblasts cultured *in vitro.* It was demonstrated that normal cells have a limited capacity for division due to telomere shortening. This phenomenon is now known as replicative senescence, although many other stress stimuli triggering cellular senescence have been discovered.

Overall, types of senescence can be grouped based on their triggers into replicative senescence, DNA damage-induced senescence, oncogene-induced senescence, oxidative stress-induced senescence, mitochondrial dysfunction-associated senescence, epigenetically induced senescence, paracrine senescence, and endocrine senescence (induced by the inflammatory environment generated by senescent cells themselves) (12). DNA damage such as telomere shortening and single- and double-strand breaks, oncogenic mutations (eg, Ras, Myc, B-Raf) that typically affect genes involved in cell cycle control, reactive metabolites like reactive oxygen species (ROS) and bioactive lipids, signals of elevated mitogens and nutrients that increase the mammalian target of rapamycin (mTOR) activity, proteotoxic stress such as protein aggregation and unfolded proteins, and damage-associated molecular patterns (DAMPs), among others, have been found to induce senescence. Most of these findings have been demonstrated in cell culture experiments and have been considered inducers of senescence *in vivo* ever since (13).

All these effectors contribute to widespread changes in gene expression, metabolism, and chromatin organization behind the growth arrest associated with senescence, structural changes in cells, and a specialized secretory activity known as the senescence-associated secretory phenotype (SASP). Additionally, it generates an inflammatory environment. Structural changes described in senescent cells include flattened, vacuolated, and enlarged morphology, altered composition of the plasma membrane, and accumulation of lysosomes and mitochondria (14).

PHYSIOLOGY AND PATHOPHYSIOLOGY OF SENESCENCE

The general objective of senescence is the elimination of unwanted cells in the body. Therefore, its involvement in cancer defense is vital: in response to oncogenic agents, senescence is induced, and replication of cancer cells is halted (the proapoptotic SASP can even eliminate surrounding tumor cells) (3,10). Under physiological conditions, it plays a key role in the response to damage or stress and in tissue repair and remodeling, such as in wound healing processes (15). Its physiological role in embryonic development is well-established, as it participates in the formation of various anatomical structures during organogenesis and regulates the proportion of different cell types (complementing apoptosis). It also occurs in healthy adult tissues as a mechanism for the maturation of megakaryocytes and syncytiotrophoblasts in the placenta and for organism protection (12,13).

When senescent cells are not efficiently eliminated and accumulate, far from promoting regeneration, they exacerbate tissue dysfunction and contribute to the genesis of diseases (16,17). Senescence not only occurs with age but also drives aging by synchronizing it in different tissues and systems (18,19).

From the above, it can be deduced that cellular senescence is a complex phenomenon that can act as a *defense* mechanism to halt disease progression (3) under physiological conditions, while in other situations, it may promote *disease development.* When localized and limited in time, it promotes tissue remodeling during growth or after tissue damage, but it also contributes to the decline of the regenerative and functional potential of tissues, inflammation, and tumorigenesis when pronounced or persistent, as seen in aged organisms.

SIGNALING PATHWAYS OF SENESCENCE

From a molecular perspective, cellular senescence can be understood as a cellular fate that occurs at any point in life and involves the action of external and internal inducers, transcription factor cascades, changes in gene expression, and chromatin remodeling (20). Initially, the activation of the p53/p21^{Cip1} and tumor suppressor p16^{INK4a} pathways occurs, generating a response that takes time to fully establish and is irreversible. The process is reinforced by an intracellular signaling loop that includes ROS (reactive oxygen species) linked to DNA damage responses, NFκB (nuclear factor kappa B), TGF-β (transforming growth factor beta), and GATA4 (guanine adenine thymine adenine), as well as an IL-1α, IL-6, and CCAAT-enhancer-binding protein beta (C/EBP-β) loop (5).

The senescent phenotype acquired by these cells is generally accompanied by an increased secretion of proinflammatory factors: TGF-β, NFκB, IL-1α, IL-6, IL-8, chemokines that draw and anchor immune cells, and activation of different enzymes such as metalloproteinases. Other characteristics of the senescent phenotype include nuclear expression of cell cycle inhibitors and tumor suppressors (p15, p16, p21, p27, p53, hypo-phosphorylated Rb), absence of proliferative markers like Ki67, expression of DNA damage markers, presence of senescence-associated heterochromatin foci (SAHF) in the nucleus, and accumulation of lipofuscin (20). As mentioned, senescent cells upregulate several antiapoptotic pathways known as senescent cell anti-apoptotic pathways (SCAPs): BCL-2/BCL-w/BCL-XL family pathway, PI3K (phosphatidylinositol-3-kinase)/Akt pathway, p53/p21/serpine pathway, ephrins/dependence receptor/tyrosine kinase pathway, HIF-1α (hypoxia-inducible factor 1 alpha) pathway, and HSP-90 (heat shock protein 90) pathway (21, 22), as they need to resist apoptosis to protect themselves from their own proapoptotic SASP. These pathways represent a vulnerability of senescent cells and have paved the way for the identification of the first class of drugs capable of targeting them (senolytics), as described later.

We should also mention that although the SASP is a characteristic feature, not all senescent cells develop it (10).

Identifying the senescent state is useful not only to locate these cells but, more importantly, to develop targeted therapies and assess their effects on senescent cells or the SASP (Fig. 1). In further investigations, the phenotypic characteristics and molecular biomarkers of senescence have been discovered both in cultured cells and tissues. However, these markers are nonspecific, and senescent cells are heterogeneous. Therefore, the approach to detect senescent cells *in vivo* currently involves the combination of multiple methods, as summarized on table I (13). It remains to be determined which methods would be most efficient for future clinical practice.

One of the classic markers of senescence, traditionally used *in vitro* in skeletal tissues such as skin or adipose tissue, is the increased activity of the lysosomal enzyme β-galactosidase or senescence-associated β-galactosidase (SAβGal). The reason why this increased activity is detected in senescent cells is due to the high lysosomal content present in these cells (12).

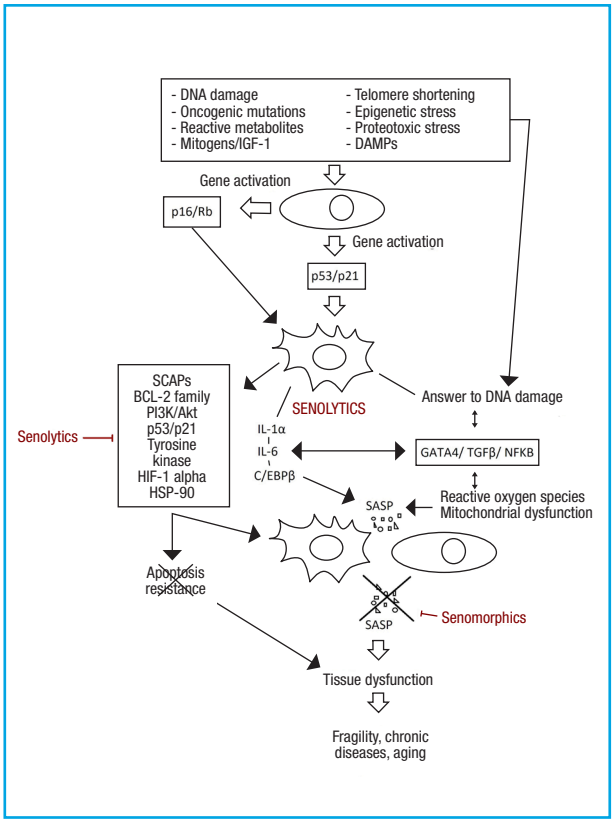

Figure 1. Inducers and mediators of senescence, SCAPs (anti-apoptotic pathways of senescent cells), SASP (senescence-associated secretory phenotype), and therapeutic targets of senotherapeutics on senescent cells. Modified from: Farr JN, et al. Bone 2019 (13).

Table I. Cellular senescence signaling at different levels		
Senescence promoters	Senescence effector genes	pl6^{Ink4}a, p21^{Cipl}, and transgenes such as pl6-LUC, INK-ATTAC [EGFP, FLAG], 3MR [mRFP]
	DNA damage	γH2AX, TAFs (co-localization of DNA damage with telomeric repeat sequences), phosphorylated p53
	Cell cycle arrest	pl6^{Ink4}a, p21^{Cipl}, DNA synthesis rate
Regulators of the senescence process	Biomarkers of inflammatory environment (SASP)	IL-6, IL-8, IL1-α, IL1-Jβ, MCP-1, Pai-1, Pai-2, MMPs, Activin A, TNFα, TGFβ, NFκβ, CEBPβ, GATA4…
	Anti-apoptosis biomarkers	SCAPs, Bcl-2, Bcl-xL, Bcl-w…
	Autophagy modulation	GDF 11 (growth differentiation factor 11)
Histological alterations	Lysosomal dysfunction	Quantity and activity of lysosomal β-galactosidase at pH 6.0
	Mitochondrial accumulation	Mitotracker staining and morphology (fusion/fission)
	Morphological alterations	Enlargement, flattening, granularity, karyomegaly, heterochromatinization, chromosomal segregation failure, elevated CCFs
	Other biomarkers	Presence of lipofuscin (GL13 staining); loss of HMGB1 and decreased laminin B1

Bcl: B-cell Lymphoma; CCFs: cytoplasmic chromatin fragments; CEBP: CCAAT/enhancer-binding protein; GATA: guanine adenine thymine adenine; HMGB1: high mobility group box 1; H2AX: histone family member X; IL: interleukin; MCP1: monocyte chemoattractant protein-1; MMP: matrix metalloproteinase; NFκB: nuclear factor kappa B; SASP: senescence-associated secretory phenotype; SCAPs: anti-apoptotic pathways of senescent cells; TNF: tumor necrosis factor; Modified from: Farr JN, et al. (13,49,50).

ASSOCIATED DISEASES

Although senescent cells can appear at any stage of life, it is known that they accumulate in tissues as chronological age increases: adipose tissue, lung, skeletal muscle, heart, kidney, bone (13,17,19). As a matter of fact, the transplantation of small amounts of senescent cells around the knee joint in young mice induces a condition similar to osteoarthritis (23). Other conditions in which the accumulation of senescent cells has been demonstrated include progeroid syndromes in children, preeclampsia, age-related macular degeneration, liver cirrhosis, cancer, and vertebral spondylosis (4,10).

Similarly, recent studies have shown that senescent cells and the SASP play a prominent role in mediating age-related conditions such as cancer, osteoporosis, frailty, cardiovascular diseases, osteoarthritis, diabetes, and obesity, among others (3,6,16).

SENOLYTICS AND SENOMORPHICS

Given the potential of eliminating senescent cells or their proinflammatory secretion to treat age-related diseases and their consequences, the detection of senescent cells and the development of therapies targeting them have become important areas of research in the biomedical field. To this date, 2 main categories of drugs are being studied: *senolytics*, which specifically eliminate senescent cells, and *senomorphics*, which suppress the SASP without inducing cell death.

The first evidence supporting the hypothesis that treating senescent cells can alleviate age-related chronic diseases came from the creation of INK-ATTAC, a transgenic mouse model in which it is possible to identify (using p16^{Ink4a}), isolate, and selectively eliminate senescent cells by administering a synthetic activating molecule: AP20187 (24). It was demonstrated that the elimination of p16^{Ink4a}-positive senescent cells improved lifespan and had beneficial effects in multiple tissues (25).

Despite the promising nature of these findings, as it involves the insertion of a transgene, this genetic approach cannot be applied to humans (26). The first class of drugs that selectively eliminate senescent cells, *senolytics*, was then identified. These are small molecules that induce cell death by apoptosis specifically in senescent cells. They work by transiently disabling SCAPs, which, as mentioned earlier, protect senescent cells from their own proapoptotic SASP. The first senolytic compounds described, first *in vitro* and then *in vivo,* were dasatinib (D) and quercetin (Q), used together (D + Q). Dasatinib is a tyrosine kinase inhibitor, and quercetin is a flavonoid found in fruits and vegetables that inhibits tumor necrosis factor alpha (TNFα) (21).

In preclinical models, senolytics have been shown to delay, prevent, and/or alleviate frailty, cancer, and cardiovascular, hepatic, musculoskeletal, and neurological disorders. Initial trials suggest that they reduce senescent cell burden, decrease inflammation, and alleviate frailty in humans. Numerous clinical trials are currently underway for various diseases to safely translate these findings into the routine clinical practice in the management of age-related degenerative diseases (10,16).

We should mention that due to the beneficial functions of senescent cells, operating on the mechanisms through which a cell becomes senescent could have detrimental effects, such as an increased risk of cancer (27-30). Therefore, the goal is to target already formed senescent cells that accumulate damage and cause tissue dysfunction through their proinflammatory SASP. The following are the most important characteristics of each group.

As mentioned before, senescent cells resist apoptosis through their SCAPs. Senolytics act by transiently disabling these SCAPs, thus leading to cell death.

If we classify senolytic agents based on the anti-apoptotic pathways they target, as shown on figure 2, they can be divided into the following categories (16,22):

- BCL-2/BCL-W/BCL-XL pathway: Navitoclax, fisetin, A1331852, A1155463.
- PI3K/Akt pathway: quercetin, fisetin, piperlongumine.
- p53/p21/serpine pathway: quercetin, fisetin, FOXO4-related peptide.
- Ephrin/dependency receptor/tyrosine kinase pathway: dasatinib (eph receptor), piperlongumine (androgen receptors).
- HIF-1α pathway: quercetin, fisetin.
- HSP-90 pathway: tanespimycin, alvespimycin (this pathway was discovered later) (31).

Recently, it has been discovered that mitoTAM (mitochondria-targeted tamoxifen) acts as a senolytic drug, and it is postulated to be part of a new group of agents targeting mitochondria, which is still not fully understood (16).

Among the first batch of senolytics discovered (10), using the aforementioned approach, we find the following ones: dasatinib, quercetin (21), fisetin (32,33), luteolin, curcumin, curcumin analog EF24, navitoclax (ABT263) (34,35), A1331852, A1155463, geldanamycin, tanespimycin, alvespimycin, piperlongumine (36), FOXO4-related peptide (37), nutlin3a, ouabain, and proscurcinidin. Some of them are natural compounds, while others are synthetic small molecules (22).

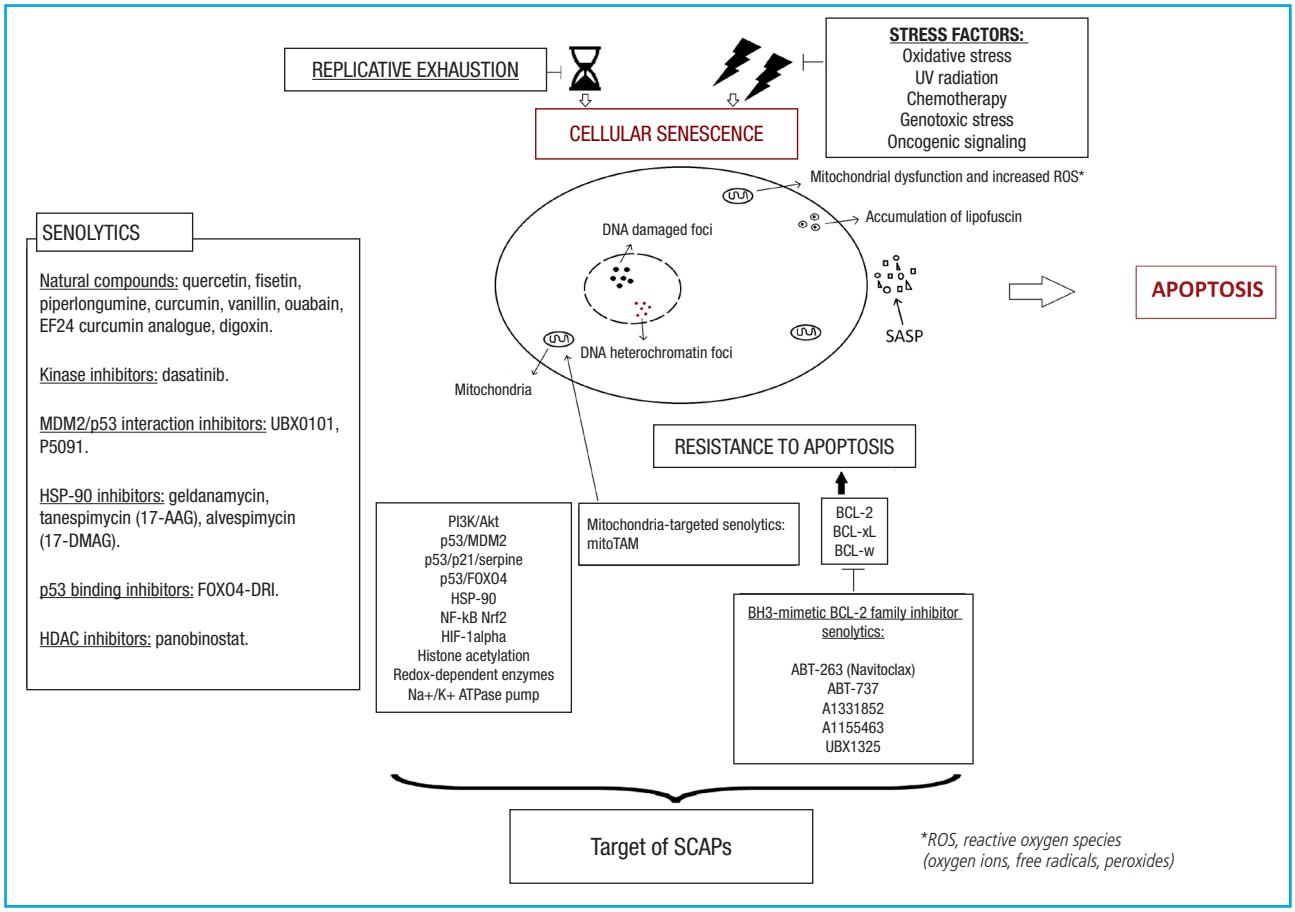

Figure 2. Main pathways targeted by senolytics. These include the BCL-2 family pathway, PI3K/Akt pathway, p53/p21/serpine pathway, ephrin/dependence receptor/tyrosine kinase pathway, and HIF-1α pathway. Modified from Lagoumtzi, et al. Free Radic Biol Med 2021 (16).

Currently, methods for identifying senolytic drugs include random screenings of drug libraries, the use of nanotechnology, or immunomodulators (10).

From the perspective of finding drugs that are as specific as possible, it is interesting that they can target multiple pathways, which reduces the chances of off-target effects. A specific case that illustrates this issue is Navitoclax (34,35), that acts on a restricted range of senescent cells but has apoptotic effects on non-senescent cells. Therefore, its clinical use is limited due to side effects such as severe thrombocytopenia and neutropenia (30).

The SCAPs necessary to survive apoptosis differ among different types of senescent cells, making it challenging to find a single senolytic that is effective against all of them (4,22,30).

Senolytics can act synergistically (4,22), as is the case with D + Q, thus opening up the possibility of expanding their spectrum of action by combining different molecules, similar to antibiotics.

Since senescent cells take time to accumulate again in a tissue and acquire a SASP, administering senolytic drugs intermittently may be enough to achieve the therapeutic goals intended, thus minimizing side effects and the risk of effects beyond the intended site of action, and allowing administration during periods of good health (21). Additionally, since senescent cells do not divide, it is unlikely that these drugs will generate resistance. Regarding dosage, it will likely depend on the circumstance leading to senescence, which will determine a specific accumulation rate for each process (4,22,38).

Prior to clinical trials in humans to demonstrate the effectiveness of a senolytic drug, it is important to make sure that the effects seen are due to its intended action and not to off-target effects. Modified Koch's postulates (4) have been proposed and are fulfilled in mice treated with D + Q for various diseases. There are also indications of their fulfillment for fisetin, although there are more doubts surrounding Navitoclax (10).

As mentioned earlier, regarding the treatment of senescence, there is another category of drugs called *senomorphic* or *senomodulating* agents, which are small molecules that act indirectly on senescent cells by inhibiting their inflammatory SASP.

Some of these agents include resveratrol, apigenin, kaempferol, metformin, glucocorticoids, rapamycin, everolimus, ruxolitinib, or EGCG (epigallocatechin gallate). Among them, some are natural compounds, while others are approved drugs for specific indications by regulatory agencies. However, it remains to be seen whether dosages would be similar. Additionally, there are molecules identified as potential senomorphic agents, such as loperamide (4,16).

The targets of these drugs are the pathways through which the SASP is expressed or acts (Fig. 3). The most important pathways, along with some notable agents that act on them, are (16):

- Inhibition of NFκB pathway: resveratrol, apigenin, kaempferol, metformin, glucocorticoids.
- Inhibition of mTOR pathway: rapamycin, everolimus.
- JAK (janus kinase)/STAT pathway (JAK inhibition): ruxolitinib.
- Antibodies against the activity and function of specific SASP mediators such as IL-6 or IL-8.

BONE SENESCENCE

The role of senescence in age-related bone loss has recently been the subject of numerous investigations. With aging, bone remodeling is disrupted, thus leading to an imbalance between the amount of new bone formation and bone resorption, resulting in a negative balance that, over time, leads to osteoporosis.

Aging manifests in the bone as a decrease in bone tissue itself and an increased marrow fat. It is not clear whether marrow fat has a direct negative effect on bone formation, although it is likely because mesenchymal stem cells (MSC) are precursors to both marrow adipocytes and osteoblasts, thus suggesting a shift towards the adipogenic lineage that, along with adipokines, may contribute to the described situation.

While the exact processes thar cause these age-related changes are still not fully understood, it has been hypothesized that the basic mechanisms of aging including senescence, are responsible for age-related bone dysfunction (13).

For years, it has been impossible to demonstrate that senescent cells accumulate in bone with aging, as seen in other tissues. It was also unknown which cells in the bone microenvironment become senescent with age and whether they are capable of generating SASP (4). In the first study that addressed these issues (39), senescence and SASP markers were measured *in vivo* in young mice (both male and female) and compared to old mice, in highly enriched populations of various cell lineages. It was discovered that the expression of $p16^{Ink4a}$ consistently increased with aging in B cells, T cells, myeloid cells, osteoprogenitors, osteoblasts, and osteocytes. Additionally, it was found that $p21^{Cip1}$ levels increased with aging in isolated cells from males enriched with osteocytes (while no change was seen in females). These findings in mice were validated in humans through the obtention of bone biopsies from elderly and young women, which revealed an age-related increase in both $p16^{Ink4a}$ and $p21^{Cip1}$. Finally, it was demonstrated that SASP is mainly produced by senescent osteoprogenitors, senescent myeloid cells, and senescent osteocytes.

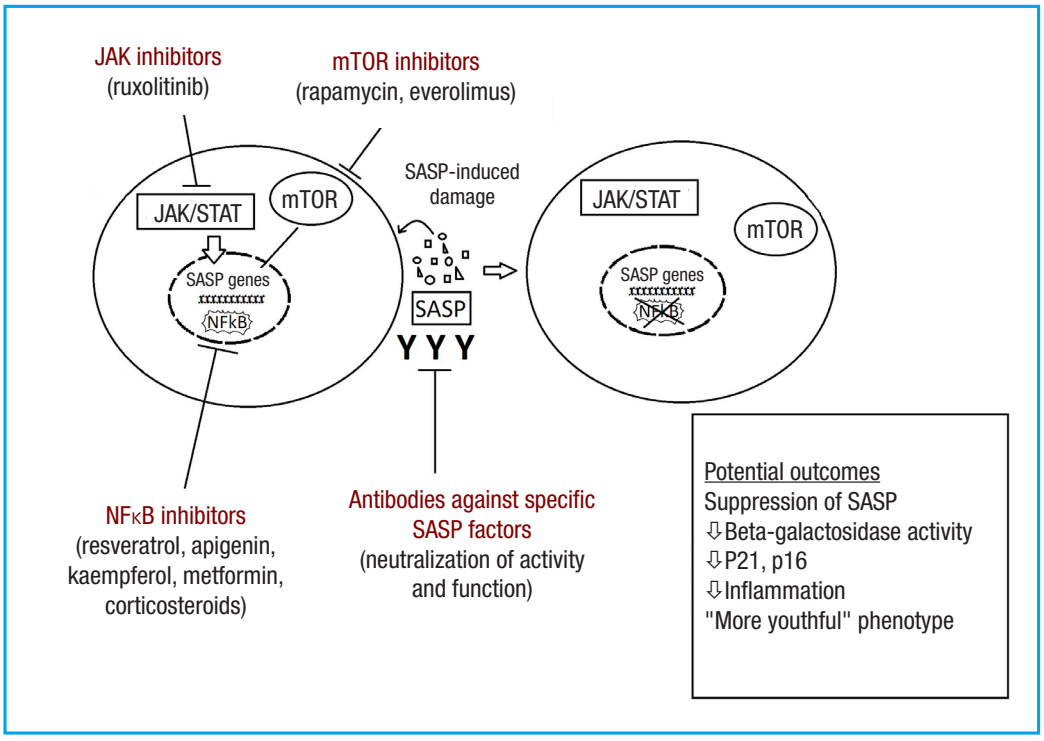

Figure 3. Main pathways targeted by senomorphics. These include the NFκB inhibition pathway, mTOR inhibition pathway, JAK/STAT pathway, and antibodies against specific SASP mediators. The mTOR kinase is key in cellular metabolism, controlling cellular catabolism and anabolism, determining whether cells (particularly cancer cells) should grow and proliferate. Additionally, mTOR has effects on apoptosis regulation. NFκB is widely used by eukaryotic cells as a regulator of genes that control cell proliferation and survival. The JAK/STAT signaling pathway is involved in processes such as immunity, cell division, cell death, and tumor formation. Modified from Lagoumtzi, et al. Free Radic Biol Med 2021 (16).

Although there are still significant gaps in knowledge and many unanswered questions, cellular senescence appears to be a global characteristic of natural aging in the bone, as in the rest of the body. This opens up the possibility that targeting senescence specifically could reduce the impact of a disease such as osteoporosis.

To determine if cellular senescence plays a role in age-related bone loss, 3 strategies were used: a genetic approach, a pharmacological approach to eliminate senescent cells (INKATTAC or senolytics, D + Q), and a senomorphic approach (using a JAK inhibitor, ruxolitinib) to inhibit SASP (26). All 3 interventions demonstrated prevention of bone loss in old mice, and none had effects on bone parameters in young mice, which is indicative of their specificity for aging (25).

In conclusion, studies on senescence in bone demonstrate that with aging, cells in the bone microenvironment (at least a subset of most cell types) become senescent and develop a heterogeneous SASP. Furthermore, they establish that senescent cells play a causal role in age-related bone loss, which can be alleviated in old mice by reducing the genetic or pharmacological burden with the first class of senolytics or with a senomorphic approach. This could represent a new strategy for treating or preventing osteoporosis, with potential advantages over conventional therapy (25,26).

Bisphosphonates are drugs commonly used in clinical practice to treat osteoporosis. One of them, zoledronic acid, could be considered a senotherapeutic agent. Animal experiments have shown that zoledronic acid extends cellular survival and delays senescence of mesenchymal stem cells, improves DNA repair by inhibiting the mevalonate and mTOR pathways, improves intestinal epithelial dysplasia, and prevents radiation-induced mutations (41). In 2007, an effect on the reduction of mortality regardless of the effect on the rate of fracture was observed. Since then, clinical trials, meta-analyses, and observational studies specifically designed to measure this relationship suggest that bisphosphonates (particularly nitrogen-containing ones including zoledronic acid) provide survival benefits in osteoporotic/osteopenic patients, as well as in patients previously exposed to various circumstances in intensive care, with cancer, or with heart disease (43). Recently, in women in their seventh decade of life with osteopenia, the ad-

ministration of 5mg of IV zoledronic acid every 18 months showed a clear trend towards lower rates of mortality, cancer, and cardiovascular events (44), indicative of a potential systemic senotherapeutic effect.

As a matter of fact, a review article on the effects of bisphosphonates and lifespan shows studies in which bisphosphonates exhibit a protective effect with hazard ratios ranging from 0.56 to 0.94 (43).

Although further studies are needed to confirm these effects, the results obtained so far are extremely promising in the sense that drugs already used and useful in the treatment of a prevalent disease like osteoporosis, such as zoledronic acid, may have additional benefits similar to those described. Furthermore, understanding this association of zoledronic acid with increased survival opens the door to investigating the behavior of senescent cell markers in patients receiving zoledronic acid treatment and patients from other therapeutic groups, to identify surrogate markers of survival that would simplify future studies in this field.

CONCLUSIONS

Cellular senescence is a phenomenon involved in aging and chronic diseases associated with aging including osteoporosis. Its mechanism is complex and ambivalent: while it can be beneficial in certain contexts, especially in young tissues, it can be detrimental due to accumulation and its senescence-associated secretory phenotype (SASP) in other cases, leading to tissue dysfunction or exacerbating it.

To mitigate its role in the development of age-related diseases, therapies targeting senescent cells or their SASP are being investigated. A more comprehensive study of senescence markers would allow us to study the effect of certain drugs already used clinically for other indications and with lower risk compared to novel drugs.

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Case Report

Heterotopic ossification after hip arthroplasty: role of bone SPECT/CT scintigraphy

Ana Moreno-Ballesteros[1], María de Bonilla-Candau[2], Blanca Cabaleiro-Burguillos[3], Ángel Custodio Rebollo-Aguirre[1], Elena Sánchez-de Mora[1], Amelia Jiménez-Heffernan[1]

[1]Department of Nuclear Medicine. Hospital Universitario Juan Ramón Jiménez. Huelva, Spain. [2]Department of Nuclear Medicine. Hospital Universitari Vall d'Hebron. Barcelona, Spain. [3]Department of Rehabilitation. Hospital Universitario Juan Ramón Jiménez. Huelva, Spain

Abstract

Heterotopic ossification is a limiting condition that predominantly affects the hip. Because of its association with post-traumatic/postoperative pathology, bone SPECT/CT scintigraphy has proven to be especially useful regarding differential diagnosis involving prosthetic mobilization, even in the absence of radiological abnormalities. Additionally, it is an effective tool for surgical planning, considering the degree of bone maturation and the possibility of creating biomodels using 3D printing.

Keywords:
Heterotopic ossification. Bone scintigraphy. SPECT/CT. Biomodel. 3D.

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Correspondence:
Ana Moreno-Ballesteros. Servicio de Medicina Nuclear. Hospital Universitario Juan Ramón Jiménez. Ronda Norte, s/n. 21005 Huelva, Spain
e-mail: anamoreno_ballesteros@hotmail.com

CASE REPORT

This is the case of a 55-year-old man treated with right total hip arthroplasty (THA) two and a half years ago with persistent pain and limited mobility, but without any significant abnormalities according to the X-ray-images (Fig. 1). A three-phase bone scintigraphy using 99mTc-diphosphonates was requested to assess prosthetic mobilization. The early-phase images did not show any significant changes (Figs. 2 A and B). However, in the late bone phase, increased tracer uptake was seen in the right femur proximal third (Fig. 2C, arrow) that in the SPECT/CT images (Fig. 3) was consistent with an enhanced osteogenic activity in bone islands (up to 1.8 cm) inside the soft tissues adjacent to the greater trochanter. These findings are consistent with heterotopic ossifications (HO), and rule out the presence of right THA mobilization.

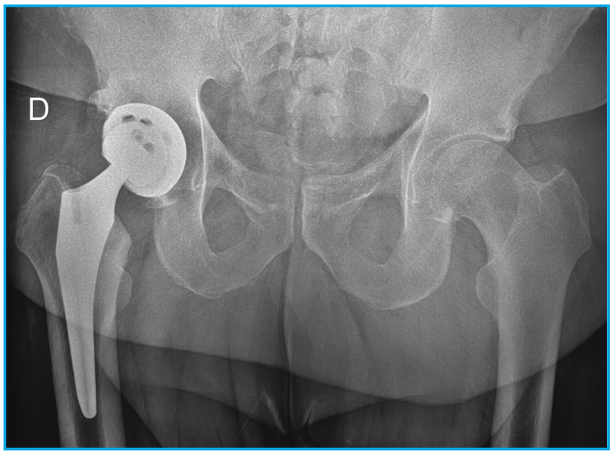

Figure 1. Plain hip X-ray after 2 years and 4 months following right THA implantation showing no signs of mobilization.

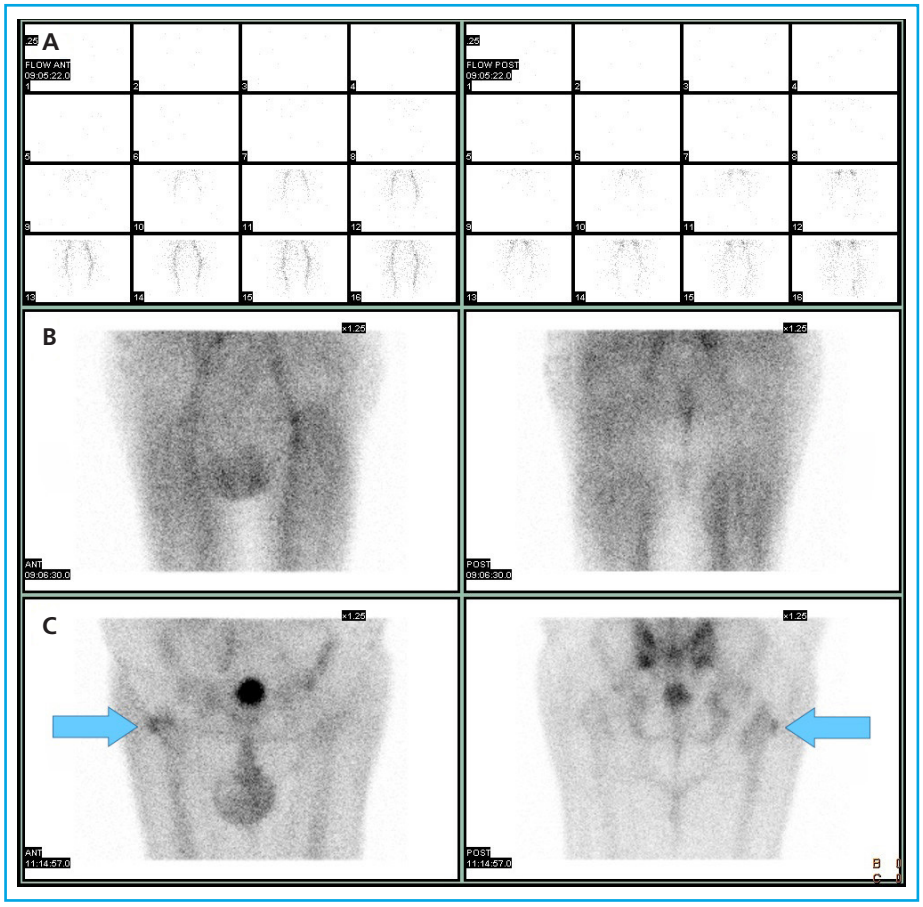

Figure 2. Anterior (left column) and posterior sections (right column) of bone scintigraphy, revealing no significant abnormalities to the early flow (A) and vascular pool phases (B). In the late bone phase (C), a focal and irregular increase in osteoblastic activity can be identified in the right femur proximal third (arrows).

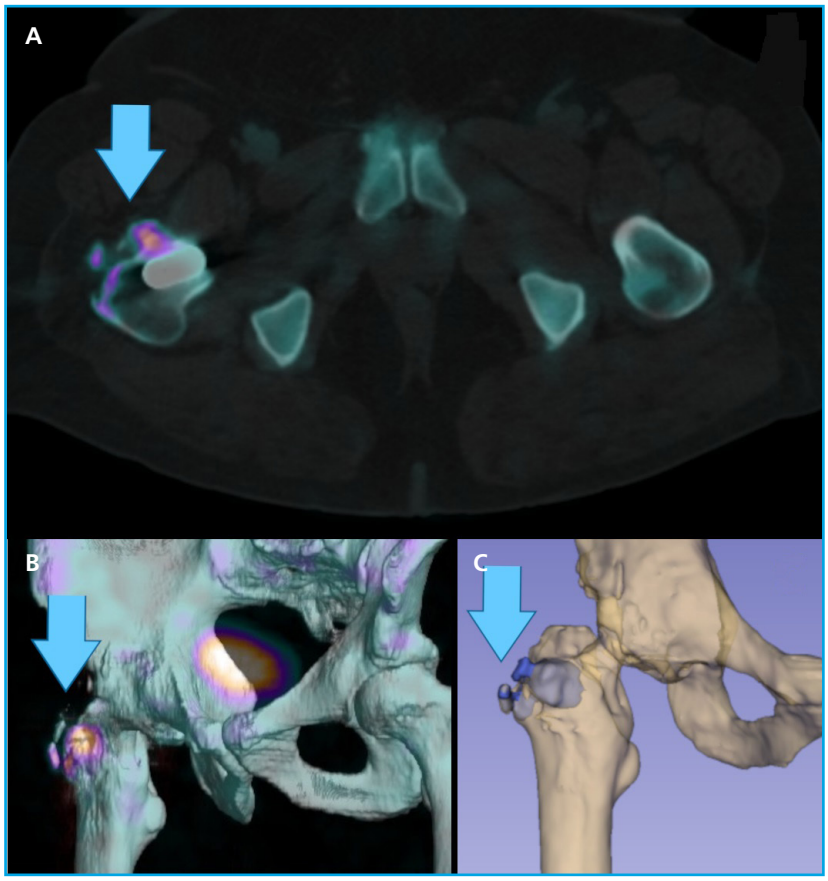

Figure 3. Axial slice of fused SPECT/CT image (A), 3D reconstruction (B), and segmentation using 3Dslicer software (9) (C) of heightened osteoblastic activity (arrows) in periarticular heterotopic ossification that rules out the presence of significant right THA mobilization. The semiautomatic segmentation of the osteoblastic activity area could be a useful tool for surgical planning.

DISCUSSION

HO is a limiting condition that causes pain and reduced joint range of motion due to abnormal mature lamellar bone formation inside the soft tissues adjacent to periarticular bone (1,2). Of variable prevalence (ranging from 10 % to 53 %) (3), the hip joint is the most widely affected one. Despite its uncertain etiology, HO is associated with a previous congenital/post-traumatic/post-operative pathology that activates osteoblast and chondroblast progenitor cells, leading to calcium salt deposits inside the connective tissue (4). Also, former studies show the presence of elevated serum levels of inflammatory cytokines (TNF, IL-1, IL-6, and monocyte chemotactic protein), and alkaline phosphatase during the early phases of this bone formation that are also present in post-traumatic repair processes (5). Scintigraphy allows the diagnosis of HO early even before radiographic changes become apparent, and even before the findings become evident on the CT/MRI (6). Additionally, SPECT/CT acquisition is particularly useful to differentiate HO from prosthetic mobilization and myositis ossificans, and enable surgical planning through 3D printing of biomodels (7,8). Although the early management of HO is conservative, scintigraphy can provide insights on the degree of bone maturation, thus determining the optimal timing for surgery if indicated (9).

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Letter to the Editor

Refining the categorization of osteoporotic fracture risk

Luis Imaicela Naula, Enrique López Gavilánez

AECE Research Group. The Association of Clinical Endocrinologists of Ecuador. Guayaquil, Ecuador

Dear Editor,

The right categorization of the risk of osteoporotic fracture is essential if we want to effectively direct our efforts in the prevention and management of individuals with osteoporosis at risk of fractures. In the article published by Lopez Gavilánez et al. in *Revista de Osteoporosis y Metabolismo Mineral (ROMM)* in 2022 (1), the implementation of intervention thresholds (age-specific and hybrid) in the Ecuadorian population is discussed based on an estimate of the risk of fracture using the country-specific FRAX tool. The rate of individuals above the intervention threshold (high risk) and thus eligible for treatment reached 2 %, while those eligible for bone density study and subsequent risk re-calculation reached 74 %. These rates increased when the hybrid threshold was used. A total of 31 % of women qualified for treatment and 76.3 % for bone mineral density study (1).

The UK National Osteoporosis Guidelines Group (NOGG) categorized the risk of fracture as low and high using fracture probability estimated using the FRAX tool (2). The use of these thresholds resulted in an underestimation of the risk of fracture in older age groups, leading McCloskey to suggest the use of a hybrid threshold with age-dependent intervention thresholds up to 70 years and a fixed threshold thereafter with a single fracture probability up to 90 years (2). Back in 2019, the NOGG guidelines refined the categorization of risk of fracture into "high" and "very high" to optimize treatment selection (anabolic or antiresorptive) in high-risk patients (3). The high-risk category would now be in the probability of fracture above the intervention threshold but below the upper assessment threshold. The low-risk category would remain below the intervention threshold level (4). Using this risk re-categorization, the number of women characterized as very high risk increased with age.

Adopting the methodology used by the NOGG (5), the European guidelines (3), and IOF-ESCEO (4) for risk category refinement, our group implemented the new thresholds to re-categorize the risk of fractures as high and very high in the same population of 2283 women studied by Lopez Gavilanez et al. back in 2022 (1). With age-specific thresholds, we found that 33 (1.4 %) and 12 (0.5 %) women were categorized as high- and very high-risk, respectively. However, with the hybrid threshold, 148 (21.4 %) and 69 (10 %) women were categorized as high- and very high-risk, respectively (Fig. 1). When both intervention thresholds were compared, the number of high- and very high-risk women selected increased by 4.5 times and 5.8 times, respectively.

The addition of re-categorization into high and very high risk to the national osteoporosis guidelines will positively impact treatment selection by physicians in the countries of the region.

Conflicts of interest: the authors declare no conflict of interest.

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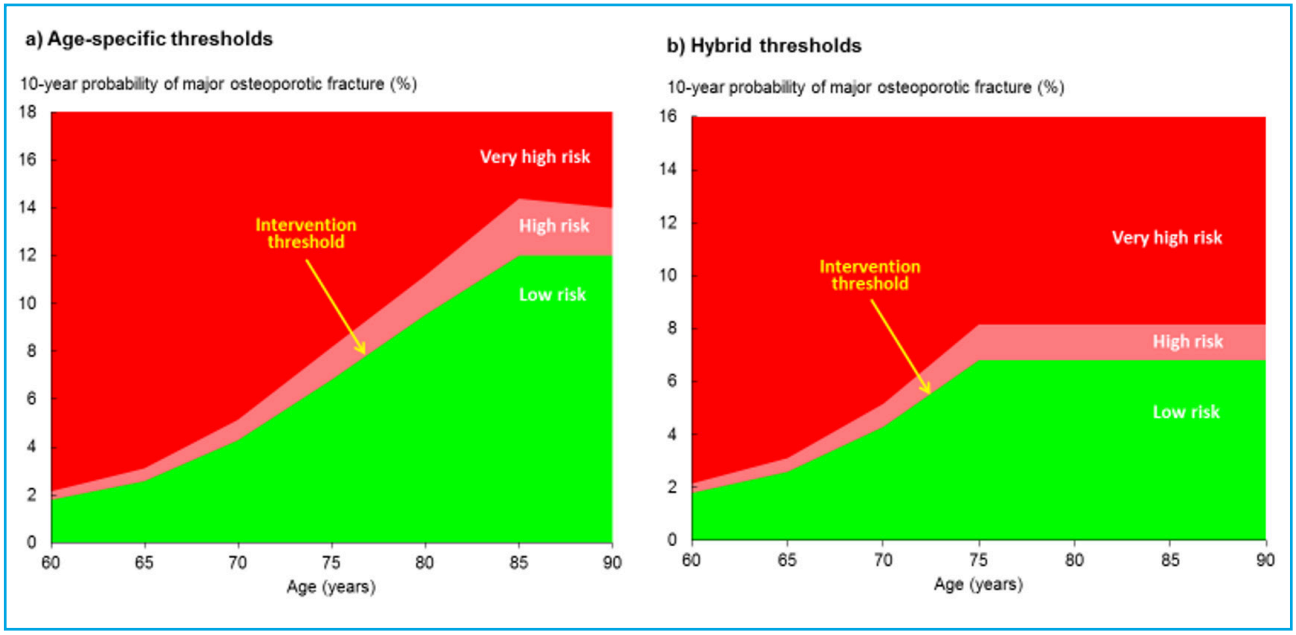

Figure 1. Fracture risk categories based on intervention thresholds. The high-risk category would now fall above the intervention threshold but below the upper assessment threshold. The low-risk category would remain below the intervention threshold level (4). Using age-specific thresholds, 33 and 12 participants were categorized as high- and very high-risk women, while with the hybrid threshold, 148 and 69 participants were categorized as high- and very high-risk women.

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